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CLINICAL STUDY PROTOCOL

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

IND No: 129492

Protocol No.: RELENT-1

Version No: Final 4.0

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This study will be conducted in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments), and in accordance with local legal and regulatory requirements.

1. PROTOCOL SYNOPSIS

PROTOCOL TITLE	EVALUATION OF THE PHARMACOKINETICS AND SAFETY OF NT-814 IN POST-MENOPAUSAL WOMEN WITH VASOMOTOR SYMPTOMS
PROTOCOL No.	RELENT-1
SPONSOR	NeRRe Therapeutics Ltd
INVESTIGATIONAL PRODUCT	NT-814
PHASE OF DEVELOPMENT	Phase 1b
INDICATION AND RATIONALE	Not applicable.
STUDY DESIGN	A multi-center, double-blind, randomized, placebo-controlled multiple ascending dose study in post-menopausal women with vasomotor symptoms. Multiple ascending doses of NT-814 (50 mg, 100 mg, 150 mg then two additional cohorts up to 400mg) will be investigated in up to 5 cohorts. Each cohort will comprise of 20 subjects randomized 3:1 (15 NT-814: 5 Placebo). No subject will participate in more than one cohort. For each cohort, subjects will enter a 4-week screening period to determine eligibility. Eligible subjects will be admitted to a Phase 1 clinical unit on Day -1 and remain in residence to receive study treatment, complete 7 days' study assessments and pharmacokinetic (PK) sampling. Subjects will be discharged on Day 8 to continue to take their allocated study treatment at home. A study visit will be performed on Day 14 at the Phase 1 clinical unit when the subjects will return for a 24 hour stay. During this visit subjects will take their final dose, complete study assessments and PK sampling, before discharge on Day 15. Single PK samples will be taken at outpatient visits on the mornings of Days 16 and 17. A safety follow-up call will take place by phone on Day 21.
STUDY OBJECTIVES Primary	• 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

Exploratory	• 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 assess 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

STUDY ENDPOINTS

Pharmacokinetic parameters	• AUC_{0-t}, $AUC_{0-\tau}$, $AUC_{0-\infty}$, Cmax, Tmax, CL/F, and t½
Safety	• Physical Examinations • Analysis for arrhythmias by continuous Holter monitoring from Day -1 to Day 8 and Day 14 (24 hours) • Abnormalities on 12-lead ECG • 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 Adverse Events (AE) • Withdrawals due to an AE • Change from baseline in ECG variables (heart rate, RR, PR, QRS, QT, QTcF and QTcB)

Exploratory	• Change in frequency of hot flashes from Pre-dose (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 paper diary throughout study • Change from Baseline in frequency of hot flashes as measured by continuous day time diary from Day -1 pre-dose to Day 7 and to Day 14 • Change from Baseline in night time awakenings (NTA) secondary to hot flashes as measured by paper diary • Change in Luteinizing Hormone (LH) $AUC_{0\text{-}8\ hours}$ 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.
PLANNED SAMPLE SIZE AND STATISTICAL CONSIDERATIONS	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 $AUC_{0\text{-}24}$ (based on Day 7 mean $AUC_{0\text{-}24}$ 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 systematic exposure. The placebo subjects are included primarily to provide a reference group for comparison of safety in the target population.
SUBJECT POPULATION	
Inclusion Criteria	1. Post-menopausal female subjects 40-65 years of age Menopause will be defined as: a) 12 months of spontaneous amenorrhea;

b) OR at least 6 weeks' post-surgical bilateral oophorectomy with or without hysterectomy.

2. At Baseline women:
   a) With an average of ≥ 7 and ≤ 20 moderate to severe hot flashes/day during the last week's diary during screening
   b) That have a change of < 50% in average 24-hour hot flash frequency between the first and second week of the screening period diary (Week -2 and Week -1 respectively).
3. Able to understand and comply with the requirements of the study and sign Informed Consent forms

Exclusion Criteria

1. Any active comorbid disease deemed by the investigator to be clinically significant which could impact safety during study conduct including renal or hepatic impairment:

   a) Serum creatinine laboratory value greater than 1.2 times upper limit of normal (ULN) reference range (after adjustment for age) at Screening or Day -1 or subjects with renal function glomerular filtration rate (GFR) <60mL/min/1.73m^2 based on the modification of diet in renal disease (MDRD) equation
   b) Total bilirubin greater than upper limit of normal reference range (with the exception of Gilbert's Syndrome) and/ or alanine transaminase (ALT) >2 times ULN reference ranges and/ or aspartate amino transferase (AST) >2 times ULN reference ranges at Screening or Day -1.
2. Subjects with a prior medical history of or an increased risk of seizures, or who have a history of recent (within 6 months of Screening) head trauma that resulted in a loss of consciousness or concussion
3. Any prior or ongoing history of arrhythmias
4. Any ongoing cardiovascular disease including heart failure, coronary artery disease or uncontrolled hypertension or uncontrolled diabetes
5. Any clinically relevant ECG abnormalities at Screening

6. Unwillingness to refrain from eating grapefruit, grapefruit juice, Seville oranges, and pomelos from 7 days prior to admission on Day -1 until after their final follow-up visit
7. Use of any prohibited medications (see Section 9.13.2)
8. Any history of invasive malignancy in the past 2 years
9. Any active ongoing condition that could cause difficulty in interpreting vasomotor symptoms such as: infection that could cause pyrexia, pheochromocytoma, hyperthyroidism, carcinoid syndrome, alcohol abuse
10. Body mass index (BMI) > 35kg/m^2
11. Inability to complete questionnaires for any reason including psychiatric disorders and inability or unwillingness to use electronic devices
12. History of hypothalamic dysfunction
13. Any clinically significant or unstable medical or psychiatric condition that would interfere with the subject's ability to participate in the study including anxiety syndromes
14. Any clinically significant abnormal laboratory test result(s) measured at Screening or on Day -1
15. Participation in any clinical research study evaluating another investigational drug or therapy within 30 days or within 5 half-lives (whichever is longer), of the investigational drug prior to consenting to study entry. If the subject is in an observational clinical study no washout is required
16. Subjects who, in the opinion of the Investigator, should not participate in the study for any other reason
17. Any known allergy or hypersensitivity to any of the ingredients in the study medication or to skin adhesives

IMP FORMULATION / DOSE	NT-814 50 mg capsules or matching placebo Up to 5 different doses will be investigated, starting with 50 mg
ROUTE OF ADMINISTRATION	Oral
DURATION/FREQUENCY OF TREATMENT	Once daily dosing for 14 days. IMP will be administered in the morning

PHARMACOKINETIC ASSESSMENTS	Blood samples for PK analysis will be collected at the following time points on Day 1, and Day 7: 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 On Day 14 blood samples will be taken for PK analysis at the following time points: 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, 48.0, 72.0 hours The pre-dose sample will be collected within 30 minutes prior to dose administration. On Days 1 and 7 the 24 hour PK sample must be taken prior to the dose of study medication the following morning.
SAFETY ASSESSMENTS	Safety and tolerability will be assessed by the following: • Physical Examinations • Continuous Holter monitoring from Day -1 to Day 8 and Day 14 (24 hours) • Abnormalities on 12-lead ECG from Day -1 to Day 8 and, Day 14 and Day15 • Change in ECG variables (heart rate, 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 BP changes • Adverse events (AE) of dehydration (serum urea and creatinine, urinary sediment, weight and vitals) • Nature and severity of AEs • Withdrawals due to an AE

EFFICACY ASSESSMENTS

Subjects will be required to complete a paper diary twice daily (morning and evening) throughout the study to record subjective hot flash severity and frequency (number of mild, moderate and severe hot flashes) and night-time awakenings. On Days -1 through to Day 7) and on Day 14 subjects will also be required to complete a paper diary on a real-time basis relating to their hot flashes. A sternal skin conductance monitor will also be fitted on Day -1 through Day 8 (24 hours post Day 7 dose) and through Day 14 to 15 (24 hours post Day 14 dose) for an objective assessment of hot flash frequency.

Blood samples for analysis of LH will be collected every 60 minutes from time 0 (pre-dose) to 8 hours on Day -1, 1 and Day 7

STATISTICAL METHODS

PK, safety and efficacy data will be presented by means of descriptive statistics and figures, as appropriate, by treatment. Data for the placebo subjects will be combined across cohorts prior to any summaries.

Plasma concentration versus time data for NT-814 will be evaluated by standard non-compartmental analysis using appropriate software. The following PK parameters for NT-814 will be log-transformed, where appropriate, prior to statistical analysis: AUC_{0-t}, $AUC_{0-\tau}$, $AUC_{0-\infty}$, Cmax, CL/F, and t½.

The safety and tolerability profile will be assessed versus baseline conditions and differences between treatment groups and descriptive statistics will be produced, where applicable. A concentration-effect model may be built investigating ECG assessments versus plasma concentrations. Exploratory dose response and PK-Pharmacodynamic (PD) analyses may be conducted for the efficacy assessments.

Any statistical hypothesis tests and/or confidence intervals 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. Raw data will be listed.

PLANNED DATE OF FIRST SUBJECT ENROLLED	01-Aug-2016
PLANNED DATE OF LAST SUBJECT COMPLETED	31-Mar-2017
NUMBER OF STUDY CENTRES	4

2. TABLE OF CONTENTS

3. LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
APTT	Activated partial thromboplastin time
AST	Aspartate amino transferase
AUC	Area under the curve
BCRP	Breast Cancer Resistance Protein
BMI	Body mass index
BP	Blood pressure
Cmax	Maximum observed concentration
CL/F	Apparent clearance
CMO	Chief medical officer
eCRF	Electronic Case Report Form
CRO	Contract Research Organization
CSR	Clinical study report
$Ctrough_{24h}$	Trough concentration at 24 hours post-dosing
ECG	Electro cardiogram
eCRF	Electronic case report form
FAS	Full analysis set
FDA	Food and drug administration
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
HDL	High-density lipoprotein
HDPE	High-density polyethylene
IB	Investigator's Brochure
ICH	International Council for Harmonization
IMP	Investigational Medicinal Product

IRB	Institutional Review Board
IWRS	Interactive Web Response Services
KNDy	Kisspeptin-Neurokinin B-Dynorphin
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LH	Luteinizing hormone
MAD	Multiple ascending dose
MCV	Mean corpuscular volume
MDRD	Modification of diet in renal disease
MnPO	Medial preoptic nucleus
NK	Neurokinin
NOAEL	No-observed-adverse-effect-level
NTA	Night time awakening
OD	Once daily
OTC	Over the counter
PD	Pharmacodynamic
PET	Positron emission tomography
PK	Pharmacokinetic
QoL	Quality of Life
RBC	Red blood cell count
RO	Receptor occupancy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SOP	Standard operating procedure
SNRI	Serotonin-norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
SUSAR	Suspected Unexpected Serious Adverse Reaction
T3	Triiodothyronine
T4	Thyroxine
$T_{1/2}$	Time to half observed concentration
TBL	Total bilirubin

Tmax	Time of maximum observed concentration
TMF	Trial master file
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
WBC	White blood cell count

4. INVESTIGATORS AND ADMINISTRATIVE STRUCTURE

List of Investigators and Affiliations

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5. BACKGROUND INFORMATION

5.1 NT-814

N.B. Prior to its acquisition by NeRRe Therapeutics, NT-814's former laboratory code name was GSK1144814. The former GSK laboratory code is used in this document when referring to the studies conducted by GSK, but NT-814 is used for all development activities conducted by NeRRe Therapeutics.

In vitro binding and functional studies have demonstrated that NT-814 is a potent and selective antagonist of both human neurokinin-1 (NK_1) and NK_3 tachykinin receptors [Catalani.et al., 2011]. NT-814 was active in pharmacodynamic (PD) models for the NK_1 receptor (the gerbil foot tapping test) and NK_3 receptor (wet dog shaking behavior in guinea pigs); both in a dose dependent manner. NT-814 also shows non-sedating anxiolytic-like properties in the human threat test in the marmoset.

The brain penetrant properties of NT-814 have been confirmed in the gerbil in which receptor occupancy (RO) in the brain was demonstrated at both the NK_1 and NK_3 receptors in a dose- and concentration-dependent manner; the RO at both receptors was sustained for extended periods post-dose. NT-814 also occupied guinea pig striatal NK_1 receptors and cortical NK_3 receptors in a dose- and concentration-dependent manner. In a positron emission tomography (PET) study in baboons, NT-814 achieved high NK_1 RO at modest plasma concentrations. These studies demonstrated that NT-814 is an orally active antagonist of brain NK_1 and NK_3 receptors in vivo.

NT-814 was shown to be >100-fold selective for the human NK_3 receptor and >300-fold for the human NK_1 receptor, in comparison to a panel of 88 other non-tachykinin human receptors, enzymes and transporters.

5.2 Hot flashes in post-menopausal women

Over the next 25 years, more than 1 billion women worldwide will be older than 50 years, and up to 75% of these women will experience adverse symptoms related to menopausal transition, particularly hot flash (also called flush) vasomotor symptoms, sleep disturbances and adverse mood that have such a negative impact upon patient daily activities and quality of life [Williams et al., 2009; Joffe et al., 2010; Santoro et al., 2015].

Hot flashes are transient, but recurrent episodes of flushing, perspiration and intense sensation are one of the most common, bothersome and distressing symptoms felt by women during the transition to menopause (peri-menopause) and post-menopause periods [Pachman et al. 2010]. They are the leading cause for seeking medical attention during this particular phase of a woman's life [Williams et al., 2007], particularly with those experiencing the severest symptoms [Whiteley et al., 2013].

Current treatment options include hormone replacement therapies which are effective in reducing vasomotor symptoms in many women, but have a safety profile which include increased thrombotic and cardiovascular effects that precludes their use in many women. The only licensed non hormonal therapy is a low dose of paroxetine which has limited efficacy and also a number of unwanted safety and toleration issues limiting widespread usage.

Kisspeptin-Neurokinin B-Dynorphin (KNDy) neurons, expressing NK_3 receptors, branch extensively within the arcuate nucleus and are also linked to the medial preoptic nucleus (MnPO) of the hypothalamus, which has been identified as the control centre for thermoregulation [Boulant, 2000] that is responsive to both estrogen and ambient temperature [Rance et al., 2013]. Support for the hypothesis that KNDy neurons have a thermoregulatory role and are involved in hot flash generation has been described in a number of animal studies [Deecher et al., 2007; Mittelman-Smith et al., 2012; Rance et al., 2013]. Furthermore in humans in a randomised, double-blinded, placebo-controlled, two-way cross-over study in healthy women it has been shown that intravenous infusion of NK-B (agonist that binds the NK_3 receptor) acutely induces hot flashes in healthy women [Jayasena et al., 2015]. Modulation of the KNDy neurons by NK_3 receptor antagonism could therefore be a viable approach to develop anti-hot flash medications

In addition, given NK_1 receptor antagonism has proven to be effective in Phase 2 studies in mood [Kramer et al., 1998; Kramer et al., 2004; Ratti et al., 2011; Ratti et al., 2013a] and primary insomnia [Ratti et al., 2013b]; it is possible that the mood and sleep comorbidities associated with menopausal hot flashes could also be addressed.

Taken together, this evidence further supports the notion that the dual $NK_{1,3}$ receptor antagonism that NT-814 offers could be an efficacious treatment for vasomotor symptoms in the menopausal patient population as well as addressing important comorbidities.

5.3 Pre-clinical research

Toxicology studies have been completed with NT-814 including single dose studies in marmoset and monkey (up to 1000 mg/kg) and repeat dose studies of up to 13-weeks in rat (up to 100 mg/kg/day) and cynomolgus monkey (up to 40 mg/kg/day). In general, the findings indicated an acceptable safety profile that did not preclude entry into clinical studies with appropriate monitoring.

Genotoxicity and reproductive toxicity studies have also been undertaken. There was no evidence of developmental toxicity in the rat at doses up to 100 mg/kg/day or in the rabbit at doses up to 140 mg/kg/day.

Further details can be found in the current Investigator's Brochure (IB).

5.4 Clinical experience

To date, four clinical studies have been completed in the European Union and Australia with single total daily doses up to 250 mg and repeat doses up to 200 mg total daily dose for 29 days. Studies comprise a single dose escalation safety study including PET (MNK111321), a repeat-dose safety study with a drug interaction assessment and PET (MNK111587), a single-dose study assessing the pharmacokinetics (PK) and effect of food on a tablet formulation (MNK112891), and a single dose study assessing whether the psychomotor and cognitive effects of alcohol are exacerbated by NT-814 (tablet formulation) (MNK113476). NT-814 has been well tolerated and no safety signals of note have been identified in the 89 male and 1 female healthy volunteers exposed to date. In addition, PK parameters from the female dosed with single doses of 100 mg and 200 mg were within the range of those seen in the males.

Further details can be found in the current IB.

5.5 Rationale for this study

To date NT-814 has been tested in 90 volunteers in 4 phase 1 studies of which 15 subjects were dosed with 200 mg up to 29 days. To date only 1 female subject has been dosed. The aim of this study is to investigate the PK and safety of NT-814 in post-menopausal women with hot flashes to help inform design of subsequent studies in the target population. The target population of post-menopausal women is required to have a minimum hot flash frequency and severity requirement for entry into this study. These characteristics have been selected as it is deemed to be important to define the safety profile of NT-814 in a controlled setting in a population with similar characteristics to that to be studied in further development due to the nature of the symptoms associated with the disease under study: hot flashes are often associated with symptoms such as palpitations, tachycardias and dizziness. To help in this assessment a careful pre-dose characterization of the subjects will be carried out on before dosing on Day -1, the study will be placebo controlled and involve 24-hour ECG monitoring through the first 7 days of dosing, when steady state plasma drug levels are predicted to be reached.

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments), and in accordance with local legal and regulatory requirements.

6. STUDY OBJECTIVES AND PURPOSE

6.1 Primary objectives

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

6.2 Exploratory objectives

- 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.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Subject numbers

Up to a total of up to 100 subjects randomized across 5 cohorts (20 subjects per cohort).

7.2 Inclusion criteria

The following inclusion criteria will apply to all subjects:

1. Post-menopausal female subjects 40-65 years of age

   Menopause will be defined as:

   a) 12 months of spontaneous amenorrhea;

   b) OR 6 weeks' post-surgical bilateral oophorectomy with or without hysterectomy.

2. At Baseline women:

   a) With an average of ≥ 7 and ≤ 20 moderate to severe hot flashes/day during the last week's diary during screening

   b) That have a change of < 50% in average 24-hour hot flash frequency between the first and second week of the screening period diary (Week -2 and Week -1 respectively).

3. Able to understand and comply with the requirements of the study and sign Informed Consent forms

7.3 Exclusion criteria

The following exclusion criteria will apply to all subjects:

1. Any active comorbid disease deemed by the investigator to be clinically significant which could impact safety during study conduct including renal or hepatic impairment:
    a) Serum creatinine laboratory value greater than 1.2 times upper limit of normal (ULN) reference range (after adjustment for age) at Screening or Day -1 or subjects with renal function glomerular filtration rate (GFR) <60mL/min/1.73m^2 based on the MDRD equation.
    b) Total bilirubin greater than upper limit of normal reference range (with the exception of Gilbert's Syndrome) and/ or alanine transaminase (ALT) >2 times ULN reference ranges and/ or aspartate amino transferase (AST) >2 times ULN reference ranges, at Screening or Day -1.
2. Subjects with a prior medical history of or an increased risk of seizures, or who have a history of recent (within 6 months of Screening) head trauma that resulted in a loss of consciousness or concussion
3. Any prior or ongoing history of arrhythmias
4. Any ongoing cardiovascular disease including heart failure, coronary artery disease or uncontrolled hypertension or uncontrolled diabetes
5. Any clinically relevant ECG abnormalities at Screening
6. Unwillingness to refrain from eating grapefruit, grapefruit juice, Seville oranges, and pomelos from 7 days prior to admission on Day -1 until after their final follow-up visit
7. Use of any prohibited medications (see Section 9.13.2)
8. Any history of invasive malignancy in the past 2 years
9. Any active ongoing condition that could cause difficulty in interpreting vasomotor symptoms such as: infection that could cause pyrexia, pheochromocytoma, hyperthyroidism, carcinoid syndrome, alcohol abuse
10. Body mass index (BMI) > 35kg/m^2
11. Inability to complete questionnaires for any reason including psychiatric disorders and inability or unwillingness to use electronic devices
12. History of hypothalamic dysfunction.
13. Any clinically significant or unstable medical or psychiatric condition that would interfere with the subject's ability to participate in the study including anxiety syndromes
14. Any clinically significant abnormal laboratory test result(s) at Screening or on Day -1.

15. Participation in any clinical research study evaluating another investigational drug or therapy within 30 days or within 5 half-lives (whichever is longer), of the investigational drug prior to consenting to study entry. If the subject is in an observational clinical study no washout is required.
16. Subjects who, in the opinion of the Investigator, should not participate in the study for any other reason.
17. Any known allergy or hypersensitivity to any of the ingredients in the study medication or to skin adhesives.

Any clinical or laboratory readings that narrowly miss the exclusion criteria such as BMI, serum creatinine and liver function tests can be considered on a case by case basis for inclusion after discussion with the medical monitor.

7.4 Withdrawal criteria

A subject should be withdrawn from treatment if they meet the discontinuation criteria in Section 9.8. If a subject is discontinued at any time after entering the study the investigator will make every effort to see the subject and complete the early termination visit assessments.

Subjects may withdraw from the study at any time without stating a reason and without prejudice to further treatment. The Investigator may withdraw a subject from the study and discontinue study treatment and assessments at any time.

Early discontinuation of any subject who has given informed consent to participate will be recorded including the reason for discontinuation. The primary reason for a subject withdrawing prematurely will be selected from the following standard categories of early discontinuations:

- **Failed to meet randomization criteria**
- **Adverse Event (Adverse Reaction)**: Clinical events occurred or laboratory results are reported that in the medical judgment of the investigator are grounds for discontinuation in the best interests of the subject.
- **Withdrawal of Consent**: The subject desired to withdraw from further participation in the study. The subject is not obliged to provide any reason for withdrawal of consent, but where a reason is given this will be recorded on the eCRF.
- **Protocol Violation**: The subject failed to adhere to the protocol requirements, at the investigator's discretion e.g. the subject needs to start taking a medication from the prohibited medications list (Section 9.13.2).

- **Lost to Follow-Up**: The subject stopped coming for visits and study personnel were unable to contact the subject.

- **Other:** The subject was terminated for a reason other than those listed above, such as theft or loss of study drugs or electronic equipment or termination of study by Sponsor.

All subjects who withdraw from the study may be replaced.

8. STUDY DESIGN

8.1 Pharmacokinetic endpoints

- Pharmacokinetics of NT-814: Peak concentration (Cmax), time of occurrence of Cmax (tmax), area under the curve (AUC) from time zero (pre-dose) to the time of the last quantifiable concentration (AUC_{0-t}), AUC in the dosing interval at steady state AUC0-τ (AUC_{0-24}) after single and repeat dosing, apparent terminal phase half-life (t½), AUC from time zero (pre-dose) extrapolated to infinity ($AUC_{0-\infty}$) after single dose, and apparent clearance (CL/F).

8.2 Safety endpoints

Safety and tolerability will be assessed by the following:

- Physical Examinations
- Analysis for arrhythmias as assessed by continuous Holter monitoring from Day -1 to Day 8 (24 hours post Day 7 dose) and Day 14 (24 hours)
- Abnormalities on the 12-lead ECGs
- Change in ECG variables (heart rate, 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 Adverse Events (AE)
- Withdrawals due to AEs

8.3 Exploratory endpoints

- Change in frequency of hot flashes from Pre-dose (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 paper diary throughout study
- Change in frequency of hot flashes as measured by continuous day time diary from Day -1 pre-dose to Day 7 and Day 14
- Change from Baseline in night-time-awakenings (NTA) secondary to hot flashes as measured by paper diary
- Change in LH $AUC_{0\text{-}8\ hours}$ 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

8.4 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 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. See Figure 1 for 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 the screening period each subject will be required to complete twice daily paper diaries. Eligible subjects will demonstrate, at the Baseline visit, an average 24-hour moderate to severe hot flash frequency ≥ 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 that have a change of $\geq 50\%$ in average 24-hour hot flash frequency between the first and second week of the screening period (Week -2 and Week -1 respectively) will be excluded. 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 a Phase 1 Unit as an inpatient 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 Phase 1 Unit on Day 14. Routine safety monitoring will be carried out in a manner that will 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 (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, atrial oxygen saturation using pulse oximetry, vital signs (sitting and standing BP, heart rate, 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 (see Section 10.7) to confirm that it is safe to proceed to the next

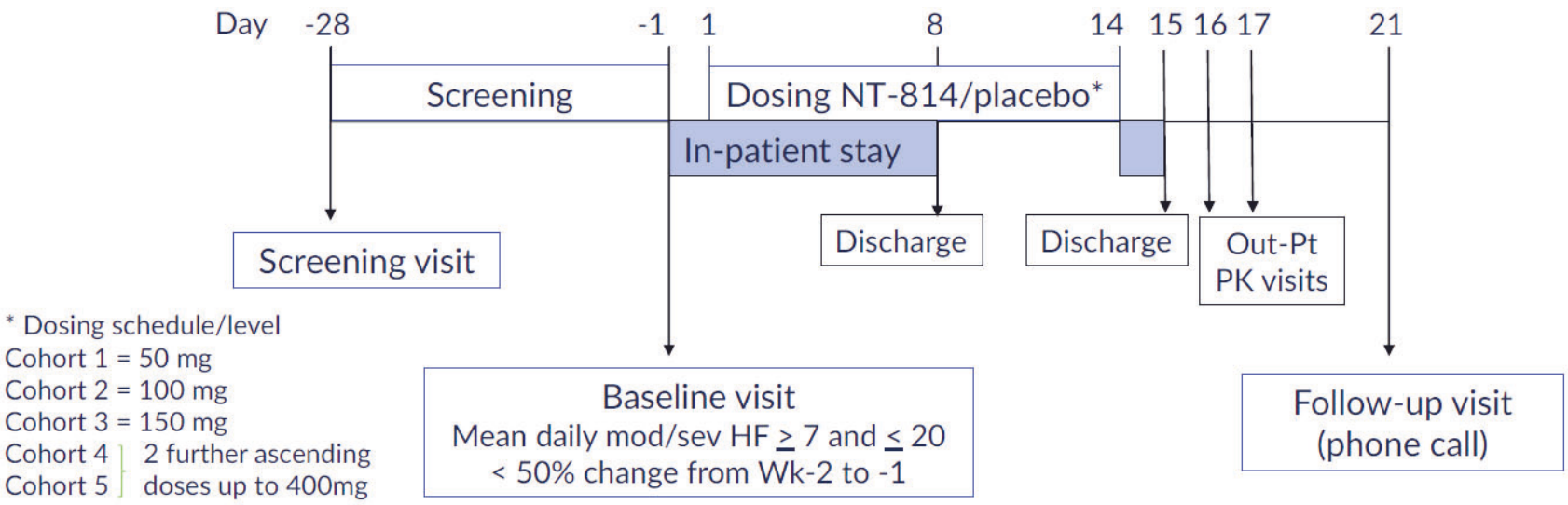
dose. This review must comprise complete data up to Day 17 from a minimum of 14 subjects per cohort.

In the case of any safety concern or any ambiguous data, from any one of the cohorts, either individual subjects or the entire cohort may be unblinded to enable decision making. In this eventuality a separate un-blinded Safety Review Committee will be used to maintain the integrity of following cohorts if needed. Details are outlined in the Safety Review Committee Charter.

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 clinic visit is deemed more appropriate by the Investigator in order to follow up any ongoing adverse events.

Figure 1: Study schematic



N.B. the data from prior cohort will be reviewed by the SRC and dosing of the subsequent cohort will only proceed if the exposure limits of a geometric mean Day 7 AUC_{0-24} of 20950 ng.h/ml are not predicted to be exceeded.

9. STUDY MEDICATION AND ADMISTRATION

9.1 Study medication

The test product, NT-814A in the free base form, formulated as a 50 mg dose in opaque white / white hard gelatin capsules to be taken orally once-daily. The physical, chemical, pharmaceutical formulation properties and characteristics of NT-814 are described in the IB.

The planned dosing schedule is as detailed in Table 1.

Table 1: Dosing schedule

Cohort 1	50 mg (1 x 50 mg capsule)
Cohort 2	100 mg (2 x 50 mg capsules)
Cohort 3	150 mg (3 x 50 mg capsules)
Cohort 4	TBD
Cohort 5	TBD

Matching placebo capsules will be used as the reference treatment in this study and will be administered as for the test product.

Subjects will take capsule(s) once a day in the morning on an empty stomach and should not eat anything for 4 hours following administration. Subjects will be required to fast from midnight prior to dosing on Days 1,7 and 14, and for up to 4 hours after (see Section 9.13.3).

Subjects will receive doses on Days 1 to 8 whilst at clinic. On Day 8, subjects will be dispensed 5 days' supply for self-administration at home on Days 9 to 13, along with an Investigational Medicinal Product (IMP) administration diary which they will be asked to complete. Subjects will be asked to return the remaining medication when they return to the clinic. The Day 14 dose will be administered in the clinic.

If subjects forget to take a dose when at home they will be instructed to miss that dose and take the next dose as scheduled.

9.2 Selection of doses in the study

To date 90 volunteers (including only 1 female) have been dosed with NT-814 with single doses up to 250 mg once daily (OD) with 15 subjects being dosed with a total daily dose of 200 mg for up to 29 days in the repeat dose human safety study (MNK111587) where attained exposures up to a

maximum AUC $_{0\text{-}24}$ of 41900 ng.h/mL were considered safe and well tolerated. A PK/PD model was created using serum total testosterone levels as a marker of target engagement from data from the Phase 1 studies. Based upon this model a mean AUC of 14040 ng.h/mL predicts near maximal target engagement at the NK_3 receptor. This exposure was achieved in male subjects following doses of 200 mg for 28 days.

The objective of this study is to characterize the PK and safety of NT-814 in a controlled setting in post-menopausal women with vasomotor symptoms to inform future development in this population using a target of maximum exposures of around 15000 ng.h/mL. To achieve the target PK AUC exposure objective, the study will be conducted in a controlled Phase 1 Unit setting with close safety monitoring and using predefined stopping criteria outlined in Section 9.9 as well as pre-defined PK stopping limits outlined below and will consist of a maximum of 4 sequentially dosed cohorts: Cohort 1, Cohort 2, Cohort 3 and if needed Cohorts 4 and 5. Planned dose escalation steps for the first 3 cohorts are in multiples of 50 mg NT-814 starting with an initial dose of 50 mg. Subsequent doses will be based on PK modelling of data from the first 3 cohorts increasing to a maximum dose of 400 mg if appropriate and needed. The predicted exposure increase between Cohorts 3 and 4 and between Cohorts 4 and 5 (if applicable) will be ≤ 2 fold. In the one-month monkey toxicity study, the effect level was considered as 60 mg/kg/day and this corresponded to a geometric mean $AUC_{0\text{-}24}$ of 35700 ng.h/mL. However, exposures up to a maximum $AUC_{0\text{-}24}$ of 41900 ng.h/mL were safe and well tolerated in the repeat dose human safety study (MNK111587); and this maximum value is used as the basis of safety margin estimates.

As outlined in the IB, the non-clinical data for sex differences in PK measures are not clear and are contradictory. Allowing for a step wise dose escalation with adequate monitoring and PK based stopping limits is considered an appropriate means of conducting the study to allow for a comparison of PK across sexes and providing for the maximum chance of achieving full target engagement.

The Cmax pharmacokinetic safety margin is based on the no-observed-adverse-effect-level (NOAEL) in the rat 3-month toxicity study. In this study GSK1144814A (vehicle, 5, 25 or 100 mg/kg/day) was given orally to rats for 13 weeks. There were no adverse, test-article related changes; therefore, the NOAEL was considered to be 100 mg/kg/day and corresponded to a geometric mean AUC of 95900 ng.h/mL and a geometric mean Cmax of 6500 ng/mL. The use of rat data to determine Cmax stopping criteria is felt to be acceptable as dose limiting toxicology in the most sensitive species (monkey) was related to total exposure (AUC) and not related to Cmax, and there have been no adverse events (AEs) of concern related to Cmax in the four Phase 1 clinical studies conducted thus far.

The study starting dose of 50 mg is anticipated to provide safety margins of approximately 12- and 14-fold respectively for $AUC_{0\text{-}24}$ and Cmax. Subjects will be dosed and monitored in a controlled Phase 1 Unit clinic setting for the first 8 days of dosing and will only be escalated to the next cohort only when safety and PK data have been analyzed.

The target exposure of 14040 ng.h/mL (geometric mean AUC_{0-24} achieved with 200 mg daily dose in males for 28 days) gives a safety margin of approximately 3-fold against the 41900 ng.h/mL observed value while the predicted geometric mean Cmax of 1836 ng/mL (at the 200 mg daily dose and after 14 days' administration) gives a safety margin of ~3.5 fold against the Cmax.

No dose will be given that is expected to exceed a geometric mean AUC_{0-24} of 20950 ng.h/mL (2-fold safety margin).

As detailed in Section 10.7, the Safety Review Committee will evaluate safety and tolerability data from each cohort prior to escalating to the subsequent dose.

If study stopping criteria (see Section 9.9) have not been met and there are no concerning safety signals, the subsequent Cohort will proceed to the next dose.

If study stopping criteria are met, no further subjects will be dosed and the study will be terminated.

In the event that study stopping criteria have not been met, but the Safety Review Committee do not feel that it is appropriate to escalate to the next planned dose, the subsequent Cohort may be dosed at any of the previous dose levels, in order to confirm safety and PK data for this dose.

9.3 Allocation to treatment

The study will be conducted in a double-blind manner, with the subjects, Investigators and Sponsor all blinded to the treatment allocated. Both NT-814 and placebo will be presented as white capsules, identical in size and shape.

Following a 4-week screening period and confirmation of eligibility, subjects in each cohort will be randomized in a 3:1 ratio to receive either NT-814 or placebo. The IMP each subject will receive will be allocated by an Interactive Web Response Services (IWRS) tool provided by the Clinical Research Organization (CRO) on behalf of the Sponsor.

The dose will start at 50 mg for Cohort 1 and will increase in increments of 50 mg for the first 3 cohorts and for subsequent cohorts, the dose will be set based on tolerability and PK as deemed appropriate by the Safety Review Committee, up to a maximum of 400 mg.

9.4 Study treatment and administration

Subjects will be administered study medication in the morning, according to the randomization schedule, at the clinic on Day 1, through Day 8. On Day 8, subjects will be dispensed study medication for self-administration 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 Phase 1 Unit. 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 clinic on Day 14. Subjects will be required to fast overnight prior to study drug administration on Day 1 through Day 14 (see Section 9.13.3).

9.5 Packaging and labelling

Investigational sites will be provided with bulk drug supplies (NT-814 50 mg capsules and matching placebo) in high-density polyethylene (HDPE) bottles packaged and labeled according to local regulatory requirements. Whilst in the clinic, doses will be prepared for the subjects each day. In-clinic doses can also be prepared in advance for convenience and stored in the secure area for dispensing each day. Upon discharge on Day 8 subjects will be provided sufficient capsules for dosing at home on Days 9 through 13, in HDPE bottles packages and labelled according to local regulatory requirements.

9.6 Handling and storage

Investigational product must be dispensed or administered according to procedures described in the Study Manual. Only subjects enrolled in the study may receive investigational product. Only authorized site staff may supply or administer investigational product. All investigational products must be stored in a secure area with access limited to the Investigator and authorized site staff. The investigational products must be kept in the original bottles and are to be stored at ambient room temperature up to maximum of 25°C. Maintenance of a temperature log (manual or automated) is required, whilst held at the study site.

9.7 Duration of subject participation

Subjects will be in the study for up to 7 weeks, from screening through to the final Follow-up phone-call assessment. During the study subjects will be required to attend the clinic on a total of 5 occasions, at Screening and for 8 continuous 24-hour assessment periods, then again on Day 14 for a 24-hour assessment, and as an outpatient for visits on the mornings of Days 16 and 17. A final Follow-up phone call will be conducted on Day 21. This will be replaced by a clinic visit if deemed more appropriate by the Investigator in order to follow up any ongoing AEs.

9.8 Discontinuation criteria

Subjects meeting any of the following criteria (regardless of treatment assignment) will be required to stop study medication and complete the early termination visit assessments:

- Intolerable complaints of undesirable sexual side effects from the study medication
- Oxygen saturation < 90% during a laboratory session that does not increase after administration of supplemental oxygen.
- If AST and ALT increase to ≥ 3 times the upper limit of normal
- Clinically relevant cardiovascular AE such as ventricular tachycardia or atrial fibrillation

- Signs of dehydration (e.g. orthostatic hypotension or decreasing urine outflow, impaired renal function or biochemistry profile change from the baseline, weight loss more than 10% from the baseline)

Subjects that discontinue early may be replaced.

9.9 Cohort and dose escalation stopping criteria

After each cohort a Safety Review Committee will review and assess all the available safety and PK study data from all of the cohorts to date in order to make a decision on the dose for the next cohort of subjects.

Data from a minimum of 14 subjects from the previous cohort must be reviewed before a decision to escalate can be taken.

The stopping criteria listed below apply to stopping during a cohort or at the end of a cohort during safety data review. Events that occur during the cohort will be passed onto the unblinded safety reviewers who will inform the study team of whether stopping criteria have been fulfilled and in such a case no other subjects will be enrolled. Subjects on treatment will be asked to stop dosing and if possible go through the early termination assessments.

1. One subject who has received NT-814 reports a serious adverse event which is considered at least possibly related to the NT-814
2. Two or more subjects, who have received NT-814, have QTc prolongation, defined as QTc > 500 ms for both Bazett and Fridericia corrections, or a prolongation from baseline of > 60 ms, confirmed (persistent for at least 5 min) during continuous ECG monitoring deemed to be at least possibly related to study drug (discovered at the end of the cohort during safety data review or on repeat ECGs taken during the study at the pre-determined time points or at the time of an AE)
3. Two or more subjects, who have received NT-814, have tachycardia, defined as resting supine heart rate > 125 beats per minute persisting for at least 10 minutes deemed to be unrelated to a hot flash episode and at least possibly related to study drug during continuous ECG monitoring (discovered at the end of the cohort during safety data review or on a repeat ECGs taken during the study at the pre-determined time points or at the time of an AE)
4. Two or more subjects, who have received NT-814, have symptomatic bradycardia, defined as resting supine heart rate < 45 beats per minute or asymptomatic bradycardia defined as resting supine heart rate < 30 beats per minute while awake persisting for at least 10 minutes during continuous ECG monitoring deemed to be at least possibly related to study drug (discovered at the end of the cohort during safety data review or on a repeat ECGs taken during the study at the pre-determined time points or at the time of an AE)

5. Two or more subjects, who have received NT-814, develop hypotension, defined as an asymptomatic fall in Systolic blood pressure (SBP) > 20 mmHg to below 70 mmHg persisting for at least 10 minutes, or a symptomatic fall in resting supine SBP > 20 mmHg (excluding vasovagal reaction or associated with hot flashes) deemed to be at least possibly related to study drug

6. One or more subjects, who have received NT-814, fulfill Hy's law, defined as "an increase in AST or ALT ≥ 3 x upper limit of normal (ULN) and total bilirubin (TBL) ≥ 2 x ULN, where no other reason can be found to explain the combination of increases, e.g., elevated serum alkaline phosphatase (ALP) indicating cholestasis, viral hepatitis, another drug. The elevations do not have to be at the same time or within a specified time frame"

7. Two or more subjects, who have received NT-814, have > 3 x ULN of either ALT or AST, or > 2 x ULN for bilirubin or ALP deemed to be at least possibly related to study drug

8. Two or more subjects, who have received NT-814, have clinical and biochemical signs suggesting dehydration as assessed by the Investigator and Sponsor (weight loss > 10% from baseline, loss of skin turgor and biochemical signs such as increased serum urea and creatinine, high urinary osmolality and sediment) deemed to be at least possibly related to study drug

9. Two or more subjects, who have received NT-814, have sustained oxygen saturations below 90% that does not increase after administration of supplemental oxygen, deemed to be at least possibly related to study drug.

10. Two or more subjects, who have received NT-814, with clinically relevant cardiovascular AEs such as ventricular tachycardia or atrial fibrillation deemed to be at least possibly related to study drug

11. Dose escalation will not occur if the exposure of NT-814 is predicted to exceed a geometric mean AUC of 20950 ng.h/mL

9.10 Premature termination of study

The study may be terminated prematurely if:

- The Investigator and the Sponsor assess that the number and/or severity of AEs justify discontinuation of the study

- The Sponsor considers the applied doses of the study drug to be no longer relevant

- The Sponsor decides to discontinue the study

- Data not known before become available and raise concern about the safety of NT-814 so that continuation would pose potential risks to the subjects

Premature termination of the study must be mutually agreed upon by the Investigator and the Sponsor and must be documented. However, study results will be reported according to the requirements outlined in this clinical study protocol as far as applicable.

9.11 Treatment accountability and compliance checks

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. Product accountability records must be maintained throughout the course of the study.

The Investigator is responsible for investigational product accountability, reconciliation, and record maintenance. The Investigator or designated site staff (e.g., storage manager, where applicable) must maintain IMP accountability records throughout the course of the study. The responsible person(s) will document the amount of investigational product received and returned (on behalf of NeRRe) and the amount administered to subjects. The accountability unit for this study is a capsule. Discrepancies are to be reconciled or resolved. Procedures for final disposition of unused investigational product will be provided in the Pharmacy Manual.

The Investigator has overall responsibility in ensuring that the study treatment is received and managed at the study site in accordance with GCP.

Limited responsibility may be delegated to a nominated study site representative and this delegation must be documented. Study treatment will be dispensed by a nominated person at each study site.

The Sponsor will be permitted upon request to audit supplies, storage and dispensing records and procedures.

9.12 Treatment blinding code

Study investigators will be given access to the IWRS system for the purposes of emergency unblinding. Investigators are permitted to unblind treatment for a subject if it is deemed that the subject's treatment will impact subject's future medical care. If possible, the Sponsor should be notified prior to the blind being broken, if not possible, the Sponsor must be notified as soon as possible if a code break was performed.

If a serious unexpected adverse reaction occurs requiring expedited reporting to the relevant regulatory agency / Institutional Review Board (IRB), then the blind will be broken for the relevant subject by the CRO safety group, in order to provide the regulators the knowledge of the event and

the causal agent. This is in agreement with ICH E2A and a blinded copy of the report will be provided to the investigators and the relevant IRB.

If unblinding occurs accidentally, this must be documented in the subject's medical notes and in the trial master file (TMF), and the Sponsor must be informed.

As detailed in Section 10.7, in the case of any safety concern or any ambiguous data, an unblinded Safety Review Committee will be permitted to review unblinded data for either individual subjects or the entire cohort to enable decision making.

9.13 Study restrictions

9.13.1 Permitted 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.

9.13.2 Prohibited concomitant medication

Subjects using the following medications will be excluded from the study:

- Any antidepressants (e.g. SSRI, SNRIs) within 4 weeks of Screening Visit
- NK receptor antagonists within 2 weeks of Screening visit
- 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, black cohosh within 2 weeks of Screening Visit
- 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 any of the following hormonal treatment before Screening Visit:
    - 1 week or longer for prior vaginal hormonal products (rings, creams, gels)
    - 4 weeks or longer for prior transdermal estrogen alone or estrogen/progestin products
    - 12 weeks or longer for prior oral estrogen and/or progestin therapy
    - 3 months or longer for prior progestin implants and estrogen alone injectable drug therapy
    - 6 months or longer 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 CYP3A4 (including but not limited to macrolide antibiotics, HIV protease inhibitor, azole antifungal drugs, cyclosporine, calcium channel inhibitor, cimetidine),
    - Inducers of CYP3A4 (including but not limited to rifampicin, carbamazepine, efavirenz, bosentan, modafinil, 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 and ritonavir, quinidine, ranolazine, verapamil).
- Known CYP3A4 substrates with narrow therapeutic range are not allowed from screening until up to 5 half-lives after last dose of NT-814 is administered (including but not limited to alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus).

As the inhibition potential of NT-814 with Breast Cancer Resistance Protein (BCRP) 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 (OTC)), including hormone replacement therapy, natural and herbal products, vitamin, mineral or dietary supplement, taken within 2 weeks (or less than 5 times the half-life of that medication, whichever is longer) prior to first dosing. Use of such medications should be reviewed with the medical monitor and permitted on a case by case basis.

9.13.3 Dietary restrictions

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.

Subjects will be required to fast from midnight prior to dosing on Days 1, 7 and 14, for up to 4 hours after. Subjects are required to continue fasting until 4 hours post dose on these days. Water will be allowed *ad libitum.* On all other dosing days, the capsules must be taken on an empty stomach and no food is to be consumed for 4 hours following administration.

9.13.4 Other restrictions

If subjects experience somnolence or drowsiness at any time after discharge on Day 8 until they return to the clinic on Day 14 they should not drive or operate heavy machinery.

10. STUDY SCHEDULE

Table 2: Schedule of Events

Procedure	Visit 1 Screen	Visit 2				Visit 3		Outpatient visits		Early term visit[12]	Follow-up Phone call[11]
	Day -28 to -1 [13]	Day -1	Day 1	Day 7	Day 8	Day 14	Day 15	Day 16	Day 17		Day 21
Informed Consent[1]	X										
Medical History/ Concomitant Diseases	X	X									
Physical Examination	X		X		X		X			X	
Inclusion/Exclusion criteria	X	X	X[2]								
Outpatient visit	X							X	X	X	
Admission		X				X					
Inpatient stay		←	—	—	→	←	→				
Discharge					X		X				
Randomization			X								
Vital signs[3]	X	X	X	X	X	X	X			X	
12-lead ECG	X	X[4]	X[4]	X	X	X[4]					
Hot flash paper diary[5]	X	X	X	X	X	X	X				
Day time continuous hot flash paper diary[5]		X	X	X		X					
Sternal Skin conductance monitor[6]		X	X	X		X					
Holter monitor[6]		X	X	X	X	X	X				
AE/SAE recording	X	X	X	X	X	X	X	X	X	X	X
Dose medication[7]			←	—	—	→					
Medication Dispensed[7]					X						
Biochemistry/ Hematology	X	X			X	X				X	
Urinalysis		X			X	X				X	
LH[8, 10]		X	X	X							
estradiol, testosterone, FSH, ACTH, cortisol, TSH, T3 and T4		X					X				
PK[9, 10]			X	X		X		X	X		

Key:

1 Informed consent must be signed by the subject before the start of any study procedure

2 Baseline assessments to be conducted on Day 1, prior to dosing. Key inclusion/exclusion criteria must be re-assessed before conducting any other baseline assessments

3 Vital signs include sitting and standing BP, heart rate, oral body temperature, pulse oximetry, respiration rate and weight will be performed daily during inpatient stays. Vital sign assessments will be performed on Day -1 at a similar time to the same assessments that are planned pre-dose on Day 1. The assessments will then be performed just prior to dosing (approx. 30 mins) for all other days in clinic and at a similar time on Day 15.

4 On Days 1 and 14, 12-lead ECG pre-dose and at 0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 12.0 and 24.0 hours (all +/- 15 mins). The same assessments schedule is required on Day -1, starting at a similar time to that planned for Days 1 and 14. Once daily ECG recordings will be made just prior to dosing (approx. 30 mins) on all other days (Days 3-8) during inpatient stays.

5 Subjects need to complete paper-diary for the 2 weeks prior to Baseline visit to ascertain eligibility. Subjects also to record number of times awoken during the night due to flashes (Night time awakening (NTA). On Days -1 to 7 and on Day 14 subjects to record the occurrence and severity of the flash (scale 1 to 3) in a continuous diary. Diary to be provided to subjects during screening and at discharge on Day 8 and subjects instructed to start completing it after waking on the mornings of Day -1 and 14 prior to admission and throughout the day and night. In the inpatient treatment part of the study, subjects also push a button on the skin conductance monitor when they sense a hot flash

6 Skin conductance and Holter monitors to be fitted on Day -1 (24 hours prior to the planned study drug administration on Day 1) and remain in place until after the 24 hour assessments are completed on Day 8 and then for 24-hours from pre-dose on Day 14 to discharge on Day 15.

7 Study drug will be administered in the morning from Day 1 to Day 14. Study medication will be dispensed to the subject on Day 8 for self-administration at home (for Days 9 to 13).

8 LH sampling every 60 mins from time 0 (pre-dose on Day 1 and Day 7) to 8 hrs. The same sampling schedule is required on Day -1, starting at a similar time to that planned for Days 1 and 7.

9 PK Sampling at 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; 48.0, 72.0 hours after Day 14 only.

10 Pre-dose samples for PK and LH to be taken within 30 minutes prior to dose administration

11 Telephone follow up visit, unless deemed by the investigator that a clinic visit is more appropriate to follow up an adverse event

12 Where possible, all subjects who are withdrawn or withdraw from the study early will undergo the Early Termination Visit assessments.

13 If the screening period is >28 days the following procedures must be repeated such that the most recent evaluation occurs within 28 days of the rescheduled randomization: biochemistry/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.

10.1 Volume of blood sampling

Each individual blood sample volume is detailed below. The volumes for individual samples may vary accordingly to local laboratory requirements, however the total volume is not expected to exceed 500 mL per subject.

Biochemistry/ hematology	6 mL
LH	3.5 mL
FSH	4 mL
PK	6 mL
Estradiol, testosterone, ACTH, cortisol, TSH, T3 and T4	4 mL

10.2 Efficacy assessments

10.2.1 Hot flash paper-diaries

Subjects will document the number of individual hot flashes experienced and rate the severity of each on a scale of 1 to 3 (mild, moderate or severe). The diaries are to be completed twice daily, in the morning and evening. The diaries are to be completed for the 2 weeks prior to Baseline visit to ascertain eligibility and from Screening until the morning of Day 15 (the last entry being the information from overnight). Subjects will also record the number of times that they were awake through the night (night time awakening [NTA]), secondary to hot flashing.

10.2.2 Day time continuous hot flash diary

Whilst in the clinic on Days -1, 1 through 7 and Day 14, subjects will record each hot flash and severity (scale of 1 to 3) in the hot flash paper-diary as they occur during the day and night. Subjects will also be required to push a button on the skin conductance monitor (see Section 10.2.3) when they sense a hot flash and then record the event in the diary. For Day -1 and Day 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 Day -1 and Day 14 prior to admission to the unit.

10.2.3 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 [Bahr et al., 2014]. 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.

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

10.2.4 Luteinizing hormone

Blood samples for analysis of LH will be collected every 60 minutes from time 0 (pre-dose) to 8 hours on Day 1 and Day 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.

LH 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.

10.3 Pharmacokinetics

Blood samples for PK analysis will be collected at the following time points on Day 1 and Day 7:

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

On Day 14 blood samples will be taken for PK analysis at the following time points:

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, 48.0, 72.0 hours

The pre-dose sample will be collected within 30 minutes prior to dose administration. On Days 1 and 7 the 24 hour PK sample must be taken prior to the dose of study medication the following morning.

Blood samples may be collected via a cannula or by direct venipuncture.

Pharmacokinetic samples will be collected as close to the nominal time point as possible. Allowable windows for PK sample collection relative to dosing (the dose at the start of the PK profile) will be as follows: ± 2 minutes up to and including 2 hours post-dose, ± 5 minutes up to and including 8 hours post-dose, ± 10 minutes up to and including 24 hours post-dose; ± 30 minutes up to and including 72 hours post-dose.

The visits on Days 16 and 17 must be scheduled to enable the samples to be taken at 48 and 72 hours, ± 30 minutes, relative to the final dose on Day 14.

10.3.1 Sample transfer and assay methodology

Plasma analysis will be performed under the management of Aptuit, SRL, Italy. Concentrations of NT-814 will be determined in plasma using the current Sponsor-approved analytical methodology. Raw data will be stored in the Good Laboratory Practice Archive of Aptuit. Please refer to the laboratory manual for further details.

Plasma concentration data will be transferred to Parexel International, USA, who will be responsible for generating the relevant tables and listings, and performing the PK analysis as described in Section 11.3.3.

10.4 Safety assessments

10.4.1 Medical history and concomitant diseases

The investigator must record all medically and clinical relevant information regardless of the time since the date of diagnosis.

History should include (but is not limited to):

- All current and past medications taken during 3 months before the Screening Visit
- Relevant history of respiratory, cardiovascular, renal, gastro-intestinal, hepatic, endocrine, hematological, neurological, psychiatric and any other diseases

10.4.2 Physical examinations

A full physical examination will be conducted at the time points indicated in the Study Schedule in Table 2. This will be completed by a physician or an appropriate qualified delegate.

A full physical examination 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 the Screening Visit will be documented on the medical history eCRF page. Any changes (including new and worsening findings) between the Screening Visit and final study visit should be captured as AEs on the AE eCRF page, as determined by the Investigator and documented in the subject's notes.

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.

10.4.3 24-hour Holter monitoring

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

10.4.4 12-lead ECGs

12-lead ECG data captured by the Holter monitors will be collected at the timepoints indicated in the Study Schedule in Table 2.

On Days 1 and 14, resting 12-lead ECG data will be collected at the following timepoints: pre-dose 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 similar time to that planned for Days 1 and 14. On Day 1 the 24 hour ECG must be performed prior to the dose of study medication the following morning.

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

These specific 12-lead ECG recordings should be collected in accordance with the priority order of study assessments detailed in Section 10.5.

10.4.5 Biochemistry

Blood for biochemistry assessments will be collected as indicated in the Study Schedule (Table 2). The following clinical chemistry/ biochemistry parameters will be assessed: sodium, potassium, glucose, urea, creatinine, creatine kinase, albumin, calcium, phosphate, bilirubin (total), alkaline phosphatase, AST, ALT, gamma-glutamyl transferase (GGT), bicarbonate, magnesium, chloride, total protein, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides.

10.4.6 Hematology

Blood for hematology assessments will be collected as indicated in the Study Schedule (Table 2). The following hematology parameters will be assessed: RBC, WBC, hematocrit, hemoglobin, MCV, platelets, WBC differentials, prothrombin time, activated partial thromboplastin time (APTT).

10.4.7 Urinalysis

Urinalysis assessments will be conducted as indicated in the Study Schedules (Table 2). Urinalysis will include glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrites, leucocytes, sedimentation.

10.4.8 Hormone level assessments

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.

10.4.9 Vital signs

Vital signs will be measured at the time points indicated in the Study Schedule (Table 2) and will include sitting and standing BP, heart rate, pulse oximetry, oral body temperature, respiration rate and weight.

10.5 Priority order of study assessments

At certain time points, several assessments will be required at the same time. In these cases, the following 'priority order' will be followed:

- Resting 12-lead ECG
- Vital signs
- PK time-points (to be collected closest to the assigned time-point)

10.6 Adverse events

In order to avoid bias in eliciting AEs, subjects should be asked a non-leading question, such as "How are you feeling?" It is also important to question the subject in a non-leading way about changes in their health or concomitant medication usage since their last visit. All AEs will be recorded from the time the informed consent is signed until study exit, and are recorded on the appropriate pages in the eCRF and in source documents. Appropriate follow-up will be required for any adverse events ongoing at study exit.

Serious Adverse Events (SAEs) occurring up to 30 days after the last dose of study medication should be reported.

10.6.1 Definitions

Adverse Event (AE)
Any untoward medical occurrence in a subject or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event (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.

Adverse Drug Reaction (ADR)
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 adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Serious Adverse Event (SAE)
An adverse event 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 (see explanation below)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is considered to be an important medical event

Based upon 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 in the definition above may be considered a serious adverse event.

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 adverse event is not an SAE.

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
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.

Unexpected Adverse Drug Reaction
An adverse reaction, the nature or severity of which is not consistent with applicable product information (e.g., IB for an unapproved investigational medicinal product).

Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events.

Suspected Unexpected Serious Adverse Reaction (SUSAR)
A serious adverse event that is suspected to be related to the administered medicinal product and the nature or severity of which is not consistent with applicable product information.

10.6.2 Assessment of causality

Severity
The severity (intensity) of each adverse event will be classified as:

- **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

Causality
An Investigator who is a qualified in medicine must make the determination of the relationship to study treatment for each AE.

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

- Unrelated
- Possibly
- Probably
- Definitely

10.6.3 Serious Adverse Event reporting

Once the Investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to the Medical Monitor within 24 hours. Any follow-up information on a previously reported SAE will also be reported to the Medical Monitor within 24 hours.

If the Investigator does not have all information regarding an SAE, he/ she will not wait to receive additional information before notifying the Medical Monitor of the event and completing the appropriate data collection form. The Investigator will always provide a preliminary assessment of causality at the time of the initial report as described in Section 10.6.2.

The primary mechanism for reporting SAEs to the Medical Monitor will be a paper collection form.

The Medical Monitor contact details for SAE receipt can be found at the beginning of this protocol on the Sponsor/ Medical Monitor Contact Information page.

The contact details for reporting SAEs are as follows:

Fax transmission or ‘scan & email’ of the SAE form is the preferred method to transmit this information to the project contact for SAE receipt. In rare circumstances and in the absence of fax equipment, notification by telephone is acceptable, with a copy of the SAE data collection form sent by overnight mail. Initial notification via the telephone does not replace the need for

the Investigator to complete and sign the SAE data collection form within the designated reporting periods

All SUSARs, which occur with the investigational products within or outside the concerned clinical trial, if required, will be reported in compliance with the applicable regulatory authorities and Institutional Review Boards (IRBs). Fatal or life-threatening SUSARs must be reported within 7 calendar days and other SUSARs within 15 calendar days.

All investigators will receive a safety letter notifying them of relevant SUSAR reports. Each Investigator must then notify his or her Institutional Review Board (IRB) of the SAE as required by local regulatory authorities and in accordance with IRB policy. NeRRe will submit to the regulatory authorities all safety updates and periodic reports, as required by applicable regulatory requirements.

10.7 Safety review committee

After each cohort, the Safety Review Committee will evaluate the safety, tolerability and PK up to 14 days of dosing with NT-814 to determine escalation to the next dose in Cohorts 2 and 3 and the doses for Cohorts 4 and 5 (if applicable). Safety data to be reviewed will include, but is not limited to, AEs, vital signs, Holter monitoring data and available laboratory parameters. This review must comprise complete data up to Day 15 from a minimum of 14 subjects per cohort. The Safety Review Committee will be comprised of the Principal Investigator, Medical Monitor, appropriate Sponsor and CRO representatives.

Following review of data from a cohort of subjects, the timing of assessments and/or blood samples may be adjusted for subsequent cohorts. Additional assessment or sampling times may be added if indicated by the data; however, the maximum blood volume taken from each subject will not exceed 500 ml.

In the event that study stopping criteria have not been met, but the Safety Review Committee do not feel that it is appropriate to escalate to the next planned dose, the subsequent Cohort may be dosed at any of the previous dose levels, in order to confirm safety and PK data for this dose.

In the case of any safety concern or any ambiguous data, during any one of the cohorts, either individual subjects or the entire cohort may be unblinded to enable decision making. In this eventuality a separate un-blinded Safety Review Committee will be used to maintain the integrity of following cohorts. Details will be outlined in the Safety Review Committee Charter.

11. STATISTICAL CONSIDERATIONS

The primary objective of the study is to evaluate the PK and safety profile of multiple doses of NT-814 compared to placebo by daily administration of each dose for 14 days. No primary hypothesis test is pre-specified for this Phase I study.

11.1 Estimated 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 AUC0-24 (based on Day 7 mean AUC0-24 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 systematic exposure. The placebo subjects are included primarily to provide a reference group for comparison of safety in the target population.

11.2 Analysis sets

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

1. **Safety Set**: All subjects who receive at least one dose of double-blind study drug. Subjects will be analyzed according to treatment received.

2. **Pharmacokinetic (PK) Set:** all subjects who received at least one dose of active study drug and for whom the PK data are considered sufficient.

3. **Full Analysis Set (FAS):** All randomized subjects who received at least one 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.

11.3 Data analysis

More details of the proposed statistical analysis will be documented in the statistical analysis plan (SAP), which will be written following finalization of the protocol and finalized prior to the breaking of the blind. PK, safety and efficacy data will be presented by means of descriptive statistics and figures, as appropriate, by treatment. Data for the placebo subjects will be combined across cohorts prior to any summaries.

Summary statistics will be based primarily on non-missing values.

Full details on the handling of missing values, including rules applied to incomplete questionnaires (including incomplete diary entries) will be defined in the SAP.

Raw data will be listed.

11.3.1 Baseline assessment

Descriptive statistics will be performed on relevant screening and baseline data (i.e. data collected prior to the treatment administration) and on demographic characteristics for each treatment group.

11.3.2 Safety analysis methodology

The safety and tolerability profile will be assessed versus baseline conditions and differences between treatment groups and descriptive statistics will be produced, where applicable.

A treatment-emergent analysis will be done for AEs. AEs will be tabulated by MedDRA preferred term and system organ class. Tabulations by severity and drug-relatedness will also be made.

A concentration-effect model will be built investigating ECG assessments versus plasma concentrations.

Extent of exposure and compliance will be evaluated.

11.3.3 Pharmacokinetic analysis methodology

Plasma concentration versus time data for NT-814 will be evaluated by standard non-compartmental analysis using appropriate software. Individual plasma concentration versus time profiles for each subject and mean and median plasma concentration versus time profiles by dose will be generated. The following PK parameters will be log-transformed prior to statistical analysis: AUC_{0-t}, $AUC_{0-\tau}$, $AUC_{0-\infty}$, Cmax, CL/F, and t½.

For each of the parameters the following summary statistics will be calculated and tabulated by treatment: n, arithmetic mean, 95% CI (confidence interval) for the arithmetic mean, standard deviation, minimum, median, maximum, geometric mean, 95% CI for the geometric mean, and standard deviation of log-transformed data. Between-subject coefficient of variation (%CVb) will also be calculated.

For tmax, tlag, n, median, minimum, maximum, arithmetic mean, 95% CI for the arithmetic means and standard deviation will be calculated.

Accumulation and time invariance will be evaluated by comparisons of Day 1 AUC_{0-24} versus Days 7 and 14 and Day 1 $AUC_{0-\infty}$ versus AUC_{0-24} Days 7 and 14 respectively.

Dose proportionality will be assessed on Day 7 (AUC_{0-24} and Cmax).

11.3.4 Exploratory analyses

Analysis of efficacy data will be primarily descriptive. Derivations of the efficacy endpoints will be provided in the SAP. Exploratory dose response and PK-PD analyses may be conducted. Any statistical hypothesis tests and/or confidence intervals 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.

11.4 Study specific data analysis

At the end of each Cohort a blinded review of PK and safety data will be conducted by the Principal Investigator, Medical Monitor and Sponsor team to confirm whether it is considered safe to continue to the subsequent dose. Dose escalation will not occur if the exposure of NT-814 is predicted to exceed a geometric mean AUC of 20950 ng.h/mL (2-fold safety margin).

In the case of an ambiguous decision, all data may be unblinded to help with the dose escalation decisions.

12. END OF THE STUDY

The end of the study will be defined as the last subject's last visit.

13. IRB REVIEW/INFORMED CONSENT

13.1 Institutional Review Board (IRB) and relevant authorities

The final study protocol and the subject information and consent form will be approved by an appropriately constituted independent Institutional Review Board (IRB). Approval will be received in writing before initiation of the study.

Clinical Trial Authorization will be obtained prior to initiation of the study from the U.S. Food and Drug Administration (FDA).

13.2 Ethical conduct of the study

The study will be performed in accordance with the local regulations, the principals of Good Clinical Practice (GCP) as described by the International Council for Harmonization (ICH), and the ethical principles that have their origins in the Declaration of Helsinki.

13.3 Informed consent

For each study subject, written informed consent will be obtained prior to any protocol-related activities. As part of this procedure, the principal investigator or one of his associates will explain orally and in writing the nature, duration, purpose of the study, and the action of the study drug in such a manner that the subject is aware of the potential risks, inconveniences, or adverse effects that may occur. They will be informed that their medical records may be reviewed by appropriately qualified monitors of the Sponsor or Sponsor Representative, and by auditors or regulatory authorities to ensure the accuracy of the details recorded as part of the study. They will be informed that they may withdraw from the study at any time without prejudice to further

treatment. They will receive all information that is required by local regulations and ICH guidelines.

14. STUDY AND DATA MANAGEMENT

14.1 Protocol amendments

Once approved by the applicable Regulatory Authorities and IRBs, the protocol must not be amended without approval by NeRRe. Unless an amendment is required to be implemented urgently in the interests of safety, any amendments to the protocol must be authorized by the applicable Regulatory Authorities and IRB prior to implementation.

14.2 Monitoring

In accordance with applicable regulations including GCP and NeRRe or delegated CRO's Standard Operating Procedures (SOP), approved clinical research monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and NeRRe requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the eCRF will serve as the source document. A list of what is to be classed as 'source documentation' will be documented in the Study Monitoring Manual.

NeRRe will monitor the study and sites to verify that the:

- Data are authentic, accurate and complete.
- Safety and right of the subjects are being protected
- Investigational Product accountability
- SAE reporting
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP and all applicable regulatory requirements.

The Investigator agrees to allow the monitor direct access to all relevant documents.

14.3 Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, NeRRe may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/ inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the Investigator and institution agree to allow the auditor/ inspector direct access to all relevant documents and to allocate his/ her time and the time of his/ her staff to the auditor/ inspector to discuss findings and any relevant issues.

14.4 Data recording

14.4.1 Case report form

An eCRF will be used to capture subject data. Access to enter data in the eCRF will be limited to delegated and trained investigator site staff only.

100% of data in the eCRF will be source data verified by monitors.

14.5 Confidentiality

Study data will be handled with utmost discretion within the context of physician's confidentiality. On the eCRFs and other study specific documents, subjects are not identified by their names, but by a unique subject number. Names of participating subjects and data generated as a result of this study will not be passed on to unauthorized persons.

The Investigator must assure that the subjects are properly anonymized throughout the study. On all study documentation which is supposed to leave the site (i.e., to be transmitted to NeRRe, CRO or third parties), subjects will only be identified by their unique identification code and will not be referred to by name.

NeRRe may transfer some data collected during the study to a different company or regulatory authority outside of the US for the purpose of processing, review, analysis or storage. Whenever the subject's personal data is transferred, it will be kept confidential and secure, and will used only for the purpose for which it was collected.

Safety analysis samples collected during the study will be analyzed at the local hospital facility per local procedures. As such samples will not be leaving the local hospital laboratory facility; there will be no specific study requirements to make the samples anonymous. All safety samples will be destroyed after the assays have been completed.

NT-814 PK samples will be shipped to Aptuit Srl, Italy for analysis. Analysis will be conducted after each cohort with the results included in the final study report. Following completion of the analysis, all samples will be destroyed.

14.6 Retention of study data

Following closure of the study, the Investigator must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/ regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The Investigator must assure that all reproductions are legible and are a true and accurate copy of the originals, and meet accessibility and retrieval

standards, including re-generating a hard copy, if required. Furthermore, the Investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

NeRRe will inform the Investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or NeRRe, or delegated CRO's SOPs; otherwise, the retention period will default to 15 years.

The Investigator must notify NeRRe of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the Investigator leaves the site. The Investigator may not dispose of any records without prior approval from NeRRe.

14.7 Communication and publication of results

Where required by applicable regulatory requirements, an Investigator signatory will be identified for the approval of the clinical study report (CSR). The Investigator will be provided appropriate access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at NeRRe or another mutually agreeable location.

The original eCRFs and all data generated during the study under this protocol will become the property of the Sponsor.

Upon completion of the CSR, NeRRe will ensure public disclosure of the clinical trial research results according to the NeRRe's SOP and provide the Investigator with the full summary of the study results. The Investigator is encouraged to share the summary results with the study subjects, as appropriate.

It is the intent of all parties that the results of the study be published in a timely manner consistent with academic standards and with due consideration given to the protection of intellectual property rights. The Principal Investigator and NeRRe will be responsible for assembling the proposed publication; this must happen with due diligence and with minimal delay.

Any proposed publication or presentation (including a manuscript, abstract or poster) for submission to a journal or scientific meeting should be sent to the Sponsor for review at least one month prior to submission. The Sponsor's comments on the proposed publication shall be considered in good faith by the authors. Sponsor may delay such submission by a maximum of ninety (90) days if it reasonably believes that publication of results may compromise its intellectual property rights or else insist that such information or data is removed from the proposed publication. Publication of the results will not include confidential information without the permission of the Sponsor.

14.8 Indemnification

In the event of study-related damage or injuries, the clinical trial insurance of the Sponsor provides compensation for claims that arise in accordance with the regulatory requirements of the countries involved, except for claims that arise from willful misconduct or gross negligence. A copy of the country-specific insurance certificates will be held in the TMF and in the Investigator site file.

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16. SIGNATURES AND AGREEMENT WITH THE PROTOCOL

Sponsor Approval

I have rev PPD rotocol follows GCP.

Signature Date: 14 December 2016

Signature Date: 14 DECEMBER 2016

Principal Investigator Approval

I agree to conduct the study according to the terms and conditions of this protocol, current Good Clinical Prac[redacted] irements. All information pertaining to the study shall be

PPD

Signature: _____ Date: 19 Dec 2016

Name of Principal Investigator
Title

Principal Investigator Approval

I agree to conduct the study according to the terms and conditions of this protocol, current Good Clinical Practice guidelines and regulatory requirements. All information pertaining to the study shall be treated as confidential.

Signature: PPD Date: 12/15/16

Name of Principal Investigator: PPD

Title P.I.

Principal Investigator Approval

I agree to conduct the study according to the terms and conditions of this protocol, current Good Clinical Practice and with applicable regulatory requirements. All information pertaining to the study shall be treated in a confidential manner.

Signature: PPD Date: 15/DEC/2016

Name of Principal Investigator

Title PPD