STATISTICAL ANALYSIS PLAN

TM004

A Phase 1 Study to Examine Pharmacodynamic Interaction Between Tesofensine and Metoprolol on 24-hours Mean Heart Rate

Version: Final 1.0

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REVISION HISTORY

Version	Version Date	Author	Summary of Changes Made
Draft 1.0	19/Sep/2018	Kees Duineveld	New Document
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Draft 4.0	07/Jun/2019	Kees Duineveld	Updates after sponsor review
Final 1.0	08/Jul/2019	Kees Duineveld	finalized

SIGNATURE PAGE – SANIONA A/S

Declaration

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

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Declaration

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

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

Document prepared and approved by:

	09 Jul 2019
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	08-Jul-19
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TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATION AND ACRONYM LIST

Abbreviation / Acronym	Definition / Expansion
AE	Adverse event
AN(C)OVA	analysis of (co-)variance
ATC	Anatomical therapeutic chemical
AUC	Area under the concentration-time curve
AUC_{0-24}	AUC from time zero to 24 hours
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BLQ	Below the lower limit of quantification
BMI	Body Mass Index
BP	Blood Pressure
Bpm	Beats per minute
CI	Confidence interval
CSP	Clinical Study Protocol
C_{max}	Maximum observed concentration
CS	Clinically significant
C_{trough}	Trough concentration in a dosing period
CV%	Coefficient of variation
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EoS	End-of-study
GAD-7	Generalized Anxiety Disorder Assessment
gCV%	Geometric CV%
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
HRmax	Maximum HR over 24 hours
HRmin	Minimum HR over 24 hours
HR24	Heart rate over 24 hours
IMP	Investigational Medicinal Product

Abbreviation / Acronym	Definition / Expansion
LLOQ	Lower limit of quantification
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
M24HR	Mean HR over 24 hours
NCS	Not clinically significant
NK	Not known
PD	Pharmacodynamic
PHQ-9	Patient Health Questionnaire-9
PK	Pharmacokinetic
QCD	Quantitative Clinical Development
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SD	Standard deviation
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
TLFs	Tables, Listings and Figures
t_{max}	Time corresponding to occurrence of C_{max}
WHO-DD	World Health Organisation - Drug Dictionary
WNL	WinNonlin

STATISTICAL ANALYSIS PLAN

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

The analyses described are based on the final CSP, 2.0 that incorporates Amendment No. 1, dated, 14/Jun/2018.

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

1. STUDY OBJECTIVES

1.1 Primary Objective

- To determine the optimal dose of metoprolol to mitigate the effect of tesofensine on Heart Rate (HR)

1.2 Secondary Objectives

- To confirm that addition of metoprolol mitigates the effect of tesofensine on HR
- To examine the concentration-response relationship between tesofensine and metoprolol on HR and blood pressure (BP)
- Assess the time dependence of tesofensine effect on HR by comparing HR data under tesofensine alone
- Safety and tolerability of tesofensine alone and co-administration of tesofensine and metoprolol

2. STUDY DESIGN

This is a randomized, open-label for tesofensine and single-blind for metoprolol, parallel-arm study in 60 male and female subjects.

Each subject will participate in a Screening Period (Day -28 to Day -3), a Baseline Period (Day -2 to Day -1) and a Treatment Period (Day 1 to Day 24) and will have 2 Follow-up phone calls (Day 30 and Day 50).

The Treatment Period will comprise:

- An initial in-house stay consisting of loading dose of tesofensine followed by a home dosing-period to Day 12
    - In Cohorts 1 and 2 the initial in-house stay is until the last loading dose of tesofensine has been administered on Day 3
    - In Cohort 3 the initial in-house stay until the third loading dose tesofensine has been administered on Day 3, followed by ambulatory visits on Days 4 and 5 with administration of the 4th and 5th loading dose with an ambulatory visit in the clinical unit on Day 7
- A second in-house stay (Day 13 to Day 24). Safety evaluations will be performed on Day 30 and Day 50 (end-of-study [EoS]) after last dosing over the phone.

A total of 60 subjects will be randomly assigned to one of the following treatment cohorts:

- Cohort 1 (20 subjects): tesofensine 0.25 mg once daily for 23 days (loading dose of 1.0 mg for the first 3 days)
- Cohort 2 (20 subjects): tesofensine 0.50 mg once daily for 23 days (loading dose of 2.0 mg for the first 3 days)
- Cohort 3 (20 subjects): tesofensine 0.75 mg (0.25 mg + 0.50 mg) once daily for 23 days (loading dose of 2.0 mg [4 tablets of 0.50 mg] for the first 5 days)

In addition, each subject will receive a single dose of 25 mg, 50 mg or 100 mg metoprolol ER in random order in the morning of Day 15, Day 18 and Day 21, respectively, within 30 minutes after start of a standard breakfast and together with the tesofensine dose, according to a Williams design.

Additional details can be found the CSP.

3. ENDPOINTS

3.1 Primary Endpoint

The dose of metoprolol which will result in no change relative to baseline in Mean HR over 24 hours (M24HR) for each respective dose of tesofensine – this will be calculated from a dose response relationship with the log-dose of metoprolol as independent variable and change in M24HR induced by various doses of metoprolol given to patients on steady-state dose of tesofensine as the dependent variable. The dose for no change in M24HR derived from above calculation and the corresponding 95% Confidence Interval (CI) will be calculated for each dose of tesofensine.

3.2 Secondary Endpoints

- The difference in increase in M24HR caused by tesofensine alone (calculated as the difference between the M24HR at pre-tesofensine baseline and the mean of M24HR on Days 14, 17, 20 and 23) and the reduction in M24HR following each of the 3 metoprolol doses (change from baseline in M24HR on Day 15, 18 or 21).
- Absolute values and Change in M24HR between pre-tesofensine baseline (Day -1) and after tesofensine alone (Day 14, Day 17, Day 20 and Day 23)
- Absolute values and Change in M24HR after tesofensine co-administered with metoprolol (Day 15, Day 18 and Day 21)
- Maximum and minimum HR over 24 hours (HR24) (HRmax and HRmin) values after tesofensine alone (Day 14, Day 17, Day 20 and Day 23)
- HRmax and HRmin values after tesofensine co-administered with metoprolol (Day 15, Day 18 and Day 21)
- Time of HRmin and HRmax after tesofensine alone (Day 14, Day 17, Day 20 and Day 23)
- Time of HRmin and HRmax after tesofensine co-administered with metoprolol (Day 15, Day 18 and Day 21)
- Mean HR during a designated quiet hour and a designated period after tesofensine alone (Day 14, Day 17, Day 20 and Day 23)
- Mean HR during a designated quiet hour and a designated period after tesofensine co-administered with metoprolol (Day 15, Day 18 and Day 21)

- HR after at least 10 minutes rest before each PK sampling time point or the corresponding time point (Day -1, Day 13 [pre-dose only], Day 14, Day 15, Day 17, Day 18, Day 20, Day 21 and Day 23)
- Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values after at least 10 minutes rest before each PK sampling time point or the corresponding time point (Day -1, Day 13 [pre-dose only], Day 14, Day 15, Day 17, Day 18, Day 20, Day 21 and Day 23)
- The following PK parameters will be determined:
- C_{trough} of tesofensine on Day 13, Day 14, Day 15, Day 18, Day 21 and Day 23
- C_{max} of metoprolol on Day 15, Day 18, and Day 21
- T_{max} of metoprolol on Day 15, Day 18, and Day 21
- AUC_{0-24} of metoprolol on Day 15, Day 18, and Day 21
- Relation between metoprolol concentration and HR will be explored by tesofensine dose
- A linear PK-PD curve for each dose of tesofensine will be generated with log value of metoprolol PK parameters (AUC_{0-24} and C_{max}) on the X-axis and change in M24HR on the Y-axis. The X-axis intercept and the corresponding 95% CI will be calculated for each dose of tesofensine

3.3 Safety Endpoints

The following safety variables will be recorded at specified time points during the study:

- Vital signs (supine BP, pulse and body temperature)
- Twelve-lead ECG: HR PR interval, QRS interval, RR interval, QT interval and QT interval corrected for HR (QTc) (Bazett's correction [QTcB] and Fridericia's correction [QTcF])
- Clinical laboratory tests (hematology, clinical chemistry and urinalysis)
- Adverse event assessments
- Concomitant medication assessments
- Physical examinations

Upon request of Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) additional assessments by cardiologist, neurologist and psychiatrist were added for safety following the early termination of the study.

4. STUDY POPULATION

The study population will consist of 60 adult subjects.

Detailed lists of inclusion and exclusion criteria are shown in Sections 4.2 and 4.3 of the CSP.

5. STATISTICAL BASIS FOR SAMPLE SIZE

No formal sample size calculation has been performed.

With a sample size of N=20 with 80% power assuming the same within-subject variability in HR as observed in the study TM001 (within-subject variability = 5%) a mean difference of 3.3 beats per minute can be detected within a tesofensine cohort. This detectable difference is smaller than the increase in HR induced by all 3 studied doses of tesofensine in the study TIPO-1; thus, 20 subjects per cohort should provide adequate sample size for the purpose of this Phase 1 study.

6. RANDOMIZATION

There will be 20 subjects in each of the 3 cohorts. Subjects will be randomly assigned to a cohort. Within each cohort the subjects will be randomized to different sequences of metoprolol doses. For 18 of the subjects within each cohort a 3 treatment Williams design will be used to determine the sequences (all six possible sequences needed). The final 2 subjects will be randomized according to parts of an extra 3 treatment Latin Square. Each metoprolol sequence will have at least one subject of each gender. To obtain the required 8 subjects for each gender, an additional 2 sequences will randomly be assigned to 2 subjects from each gender.

7. STATISTICAL ANALYSIS CONVENTIONS

7.1 Analysis Variables

7.1.1 Demographic and Background Variables

The following demographic and anthropometric information will be recorded as given in Section 9:

- Date of informed consent
- Medical history (including previous and current medical conditions and medications)
- Drug, alcohol and smoking history

- Age as provided in the database.
- Gender
- Ethnic origin
- Race
- Height, without shoes (cm)
- Body weight (kg)
- Body mass index (BMI) calculated as [weight/height^2] (kg/m^2)
- Waist Circumference
- Special Diet (vegetarian)
- History of blood or plasma donation

All medical history will be coded using Version **20.1** of the Medical Dictionary for Regulatory Activities (MedDRA).

7.1.2 Safety Variables

7.1.2.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs will be coded using Version **20.1** of the MedDRA.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins or that worsens in severity after at least one dose of the study drug has been administered.

Any AEs with incomplete start and end dates/times will be treated as follows:

- Adverse events with unknown start and/or end times (but where the date is known) will be imputed with a time of 00:00 h for the tabulations but will be shown as NK:NK in the listings (where NK = Not Known).

- Adverse events with completely unknown start dates will be considered as treatment-emergent for the tabulations and will be shown as NK in the listings, unless it is clear from supporting information that the AE is not treatment-emergent.

The following assignment to a specific treatment will be made:

- An AE is assigned to the last treatment prior to the AE
- In case of missing start dates or times and taking into account supporting information (such as end date times) then the following rules will apply.
  - If an AE can occur on either after tesofensine or tesofensine plus metoprolol, then it will be assigned to tesofensine plus metoprolol
  - if an AE can occur at two different doses of metoprolol then the lower dose will be chosen. (e.g. if an AE can be either attributed to Tesofensine + 25 mg Metoprolol or Tesofensine + 100 mg Metoprolol, then Tesofensine + 25 mg Metoprolol will be chosen, [a day between those with just Tesofensine will not be used because of the previous rule]).

7.1.2.2 Clinical Laboratory Tests

The safety laboratory parameters in Table 1 will be measured according to the schedules in Section 9:

Table 1 Clinical Laboratory Assessments

Hematology	
White blood cell (WBC) count	Neutrophils (percentage and absolute count)
Red blood cell (RBC) count	Lymphocytes (percentage and absolute count)
Hemoglobin (Hb)	Monocytes (percentage and absolute count)
Hematocrit (HCT)	Eosinophils (percentage and absolute count)
Mean corpuscular volume (MCV)	Basophils (percentage and absolute count)
Mean corpuscular hemoglobin (MCH)	Platelet count
Mean corpuscular hemoglobin concentration (MCHC)	RBC distribution width
	HbA1c (at Screening only)

Table 1 Clinical Laboratory Assessments

Clinical Chemistry	
Alanine aminotransferase (ALT)	Glucose
Albumin	Lactate dehydrogenase (LDH)
Alkaline phosphatase (ALP)	Phosphorus
Aspartate aminotransferase (AST)	Potassium
Blood urea nitrogen (BUN)	Sodium
Calcium	Total bilirubin
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Thyroid-stimulating hormone (TSH)
Creatine phosphokinase (CPK)	Uric acid
Gamma glutamyl transferase (GGT)	Follicle-stimulating hormone (female subjects only, at Screening only)
Urinalysis	
Bilirubin	Blood
Glucose	pH and specific gravity
Ketones	Protein
Leukocytes	Urobilinogen
Nitrite	
Upon a positive urine test from leucocytes, blood or protein, the Investigator may require further urine analysis, such as flow cytometry. Results of additional urine analyses will be included in the database. If the flow cytometry examination shows a different result than the urine stix, it will be checked by microscopy or full automated digital imaging, (e.g., leukocytes, erythrocytes, casts in urine will be analyzed).	
Viral Serology	
Hepatitis B core antibody (anti-HBc)	Human immunodeficiency virus (HIV) (Types 1 and 2) antibodies
Hepatitis B surface antigen (HBsAg)	
Hepatitis A virus antibodies (immunoglobulin M)	Hepatitis C virus antibody (anti-HCV)
Pregnancy Tests (in Female Subjects)	
β-human chorionic gonadotropin in serum (at Screening)	β-human chorionic gonadotropin in urine (on each admission)
Urine Drug Screening, Cotinine and Alcohol Test	
Amphetamines	Cocaine
Barbiturates	Opiates
Benzodiazepines	Phencyclidine
Cannabinoids	Cotinine
Alcohol	Urinary creatinine (to exclude dilution effect)

7.1.2.3 Vital Signs

The vital signs measurements will be obtained according to the schedules in Section 9. In case of triplicate measurements the original three measurements will be listed, while the mean will be listed and used for further analysis. The following measurements will be obtained.

- Systolic blood pressure (SBP) [mmHg]
- Diastolic blood pressure (DBP) [mmHg]
- Pulse rate (bpm)
- Body temperature (oral) [°C]
- Interpretation

7.1.2.4 Safety Electrocardiograms (ECG)

The standard safety 12-lead ECG parameters will be recorded according to the schedules in Section 9. In case of triplicate measurements the original three measurements will be listed, while the mean will be listed and used for further analysis. The following measurements will be obtained.

- RR-interval (msec)
- PR-interval (msec)
- QRS-interval (msec)
- QT-interval (msec)
- QT-interval corrected using the Bazett correction formula (QTcB) (msec)
- QT-interval corrected using the Fridericia correction formula (QTcF) (msec)
- Heart rate (HR) (beats per minute [bpm])

The ECG will be evaluated by the Investigator as 'Normal', 'Abnormal, Not Clinically Significant (NCS)' or 'Abnormal, Clinically Significant (CS)'.

7.1.2.5 Physical Examination

Physical Examination will be performed according to the schedules in Section 9.

7.1.2.6 Concomitant Medication

Prior and concomitant medication will be coded using the World Health Organisation-Drug Dictionary (WHO-DD) (Version **Sep 2017**) and will be classified by Anatomical Therapeutic Chemical (ATC) categories.

7.1.2.7 Patient Health Questionnaire-9 and (PHQ-9) and Generalized Anxiety Disorder Assessment (GAD-7)

The Patient Health Questionnaire-9 (PHQ-9) and Generalized Anxiety Disorder Assessment (GAD-7) [Ref: Instruction Manual] will be performed according to the schedules in Section 9.

PHQ-9 Depression Severity.

This is calculated by assigning scores of 0, 1, 2, and 3, to the response categories of "not at all", "several days," "more than half the days", and "nearly every day", respectively. PHQ-9 total score for the 9 items ranges from 0 to 27. Scores of 5, 10, 15, and 20 represent cut points for mild, moderate, moderately severe and severe depression, respectively. Sensitivity to change has also been confirmed.

GAD-7 Anxiety Severity.

This is calculated by assigning scores of 0, 1, 2, and 3, to the response categories of "not at all", "several days", "more than half the days", and "nearly every day", respectively. GAD-7 total score for the 7 items ranges from 0 to 21. Scores of 5, 10, and 15 represent cut points for mild, moderate, and severe anxiety, respectively.

7.1.2.8 Additional assessments based on BfArM's request

Additional evaluations by cardiologist, neurologist and psychiatrist will be displayed for normal/abnormal result, including description of abnormality, clinical significance and further action. Associated AE and Concomitant medication will be added in the database.

7.1.3 Pharmacokinetic Variables

Unless otherwise stated, derivation of tesofenesine and metoprolol PK parameters will be the responsibility of Early Phase, Quantitative Clinical Development (QCD), PAREXEL International.:

At a minimum, following PK parameters will be computed for metoprolol on Day 15, 18 and 21.

- Maximum observed plasma concentration (C_{max})
- Observed time at C_{max} (T_{max})
- Area under the plasma concentration time curve (AUC) from time zero to 24-hour post-administration of metoprolol (AUC0-24).

PK parameters computed for tesofensine will be:

- Trough concentration of the day. On days when 0 and 8 hour blood samples are drawn, the lower concentration will be considered the trough concentration, C_{trough}, of the day. On days when only 0 hour sample is drawn, that concentration will be considered C_{trough}.

7.1.3.1 Pharmacokinetic Parameter Calculation Methods

Metoprolol PK parameters will be calculated by non-compartmental analysis methods from the concentration-time data using Phoenix® WinNonlin (WNL) Professional (Version **6.3 or higher**) or SAS (Version 9.3 or later) following these guidelines:

- Actual sampling times relative to dosing rather than nominal times will be used in the calculation of all derived PK parameters.
- There will be no imputation of missing data.
- Any subjects with missing concentration data will be included in the PK analysis set provided that at least C_{max} or AUC_{0-24} of metoprolol can be reliably calculated as determined by the pharmacokineticist.
- For calculation of PK parameters, all BLQ values will be treated as missing, except for first dose pre-dose sample where it will be substituted with zero before the first quantifiable concentration.

PK parameters will be estimated according to the following guidelines:

- C_{max} will be obtained directly from the concentration-time data.
- t_{max} is the time at which C_{max} is observed.
- AUC_{0-24} will be calculated as follows:

  $AUC_{0-24} = AUC_{0-t_{last}} + AUC_{t-24}$. $AUC_{0-t_{last}}$ will be computed using trapezoidal up and log-trapezoidal down method to the last measurable concentration, C_{last}. AUC_{t-24} will be estimated by

integrating C_t*exp(-lambda*s) between t and 24h, where lambda is the terminal elimination rate. If concentrations are not BLQ at 24 hr, no extrapolation is needed.

7.1.4 Pharmacodynamic Variables

A Holter-ECG device will be used for recording of 24-hour HR at the time periods detailed in the Schedules of Assessments (Section 9).

The following PD parameters will be extracted from the Holger-ECG

- Primary endpoint M24HR is the mean HR over 24 Hours
- Minimum and maximum HR in the 24 hour period, HRmin and HRmax, also time of HRmin and HRmax
- Mean HR from a designated period (from 3 to 16 hours post-dose)
- Mean HR in a quiet hour (from 12 to 13 hours post-dose)

  During the quiet hour, subjects will have to rest, i.e., each subject will be asked to find a personally comfortable supine position with the head and whole body firmly supported. The subjects will not be permitted to watch television or to listen to radio or music player. They will not be permitted to read, to sleep or converse, and will be kept free from any external disturbance (e.g., personal media). No electric equipment including mobile phones may be switched on in the room.

In addition, Heart Rate from 12-lead ECG, BP and pulse will be assessed after a rest of at least 10 minutes as detailed in the Schedules of Assessments (Section 9).

7.2 Analysis Populations

7.2.1 Safety Population

All subjects who received at least one dose of tesofensine. Subjects will be included in the analysis according to the dose of tesofensine/metoprolol received in the actual period of interest. The safety population will be used for all safety analysis, disposition, demographics and to list PK concentrations.

7.2.2 Pharmacokinetic Population

All randomized subjects with at least one quantifiable tesofensine or metoprolol concentration and without major protocol deviations which could influence the PK. Subjects will be included in the analysis

according to the dose of tesofensine/metoprolol received. The PK population will be used for all PK analysis.

Data may/will be excluded (depending on the protocol and compound under study) from PK analysis (concentrations listed only) if any of the following criteria are fulfilled:

- Concomitant medication, which could render the plasma concentration-time profile unreliable
- The pre-dose concentration is greater than 5% of the corresponding C_{max} in any given treatment period.
- Subject vomits within 2 x the reported median t max for the analyte.
- Subject has moderate or severe diarrhea within 2 x the reported median t_{max} for the analyte.

Any data excluded will be discussed in the CSR.

7.2.3 Pharmacodynamic Population

All randomized subjects with at least HR assessments (24 Hour Holter) on Baseline (Day -2 to -1) and on at least 2 Visits out of, Day 15, Day 18 and Day 21 without any major protocol deviations which could influence the PD parameters. Additional subjects may be included during the data review meeting for purpose of PD analysis, however, these will not be included in the primary analysis. Subjects will be included in the analysis according to the dose and IMP received. The PD population will be used for all PD analysis.

7.3 Statistical Analysis Methods

7.3.1 Listings and Descriptive Statistics

All original and derived parameters as well as population characteristics will be listed and described using summary statistics. Frequency counts (number of subjects [n] and percentages) will be made for each qualitative variable. Descriptive statistics (n, mean, standard deviation [SD], median, minimum and maximum) will be calculated for each quantitative variable (unless otherwise stated). For descriptive statistics of PK variables see Section 7.3.11. All listings will include repeated and unscheduled measurements.

In case of triplicate measurements, the original three measurements will be listed together with the means, while the means will be used for summaries and baseline (see Section 7.3.2).

The following rules will apply to any repeated measurements:

- If the repeated measurement occurs prior to the first dose of study drug then the last obtained value of any repeated measurement will be used in the descriptive statistics
- If the repeated measurement occurs after the first dose of study drug then the original value of any repeated measurements will be used in the descriptive statistics
- If a triplicate is whole or partly repeated prior to the first dose of study drug, then mean of the last (part) triplicate will be used in descriptive statistics and analyses
- If a triplicate is whole or partly repeated after the first dose of study drug then the original mean value of any repeated measurements will be used in the descriptive statistics and analyses
- if more than three measurements are made in a triplicate, then all will be used

7.3.1.1 Treatments in outputs

In listings the outputs will be sorted by visit and time point. Unless otherwise described, in tables, the outputs will be sorted by treatment and time point. Summaries before any dosing and after the treatment period (screening and follow up) will be displayed without treatment. Treatments without metoprolol will be summarized by cohort, treatment (tesofensine dose), visit and time point. Treatments with metoprolol will be summarized by cohort, treatment and time point, without visit.

In particular, pre-dosing safety assessments on metoprolol dosing dates will be assigned to the dosing of the previous day, the 24 hour post dose assessment will be assigned to the Metoprolol treatment.

For PK concentration samples, see Section 7.3.11.

The following treatments will be used:

Cohort 1

- Tesofensine 0.25 mg
- Tesofensine 0.25 mg, Metoprolol 25 mg
- Tesofensine 0.25 mg, Metoprolol 50 mg
- Tesofensine 0.25 mg, Metoprolol 100 mg

Cohort 2

- Tesofensine 0.5 mg
- Tesofensine 0.5 mg, Metoprolol 25 mg
- Tesofensine 0.5 mg, Metoprolol 50 mg
- Tesofensine 0.5 mg, Metoprolol 100 mg

Cohort 3

- Tesofensine 0.75 mg
- Tesofensine 0.75 mg, Metoprolol 25 mg
- Tesofensine 0.75 mg, Metoprolol 50 mg
- Tesofensine 0.75 mg, Metoprolol 100 mg.

7.3.1.2 Analysis periods

During the treatment period the following periods will be recognized:

1. Baseline Period Tesofensine (Day -1)
2. Steady dose of Tesofensine (Day 1 to Day 14)
3. Tesofensine plus Metoprolol (Day 15)
4. Steady dose of Tesofensine (Day 16, 17)
5. Tesofensine plus Metoprolol (Day 18)
6. Steady dose of Tesofensine (Day 19, 20)
7. Tesofensine plus Metoprolol (Day 21)
8. Steady dose of Tesofensine (Day 22, 23)

7.3.2 Baseline

The baseline for all measurements (where applicable) will be the last pre-dose measurement, with exception for the PK/PD analysis, where time matched baseline will be used (see Section 7.3.13).

If a repeated measurement is the last measurement prior to the first dose of study drug, then it will be used to calculate change from baseline as applicable.

In case of triplicate measurements, the baseline and change from baseline will be obtained from the triplicate within each time point. In case of missing data within a triplicate, the remaining data will be used for the baseline. Following the rules above, if a triplicate prior to dosing is partly or fully repeated, then mean or the repeat will be used as baseline.

7.3.3 Statistical Significance Level

All statistical tests will be two-sided and will be performed at the 5% level of significance. Confidence intervals will be two-sided and be at 95% level of significance.

7.3.4 Software

All statistical analyses will be performed using Statistical Analysis Software (SAS®) Version 9.3 or later. The PK analysis will be performed using Phoenix WinNonlin Professional Software Version 6.3 or later or SAS Version 9.3 or later.

7.3.5 Missing Data

There will be no imputation of missing data, except for the calculation of PK parameters, see Section 7.1.3.1 and 7.3.11.1.

7.3.6 Interim Analysis

No formal interim analysis will be performed.

However, after completion of approximately 20 evaluable subjects, MHR24 obtained on Days -1, 14, 15, 17, 18, 20, 21 and 23 will be reviewed on an individual basis to confirm that the M24HR returned to baseline conditions are maintained prior to each metoprolol dosing. No summaries of these data will be created nor will any other data be evaluated during this interim look.

7.3.7 Protocol Deviations

All protocol deviations will be recorded by the Investigator and will be listed by subject. All protocol deviations will be discussed between PAREXEL (physician, Data Manager, Biostatistician, PK Scientist/Analyst and Medical Writer) and also the Sponsor during the clean file meeting before database lock in order to determine whether these may warrant exclusion of a subject from the pre-defined analysis populations or selected samples/measurements from the analysis.

7.3.8 Demographic Data

All demographic data will be presented using the safety population. Demographic data will be summarized by cohort and overall.

7.3.9 Concomitant Medication

Prior and concomitant medication will be listed by subject and will include the following information: reported name, PT, the route of administration, dose, frequency, start date/time, duration and indication. Prior medications are those that started and stopped prior to the first dose of IMP. Concomitant medications are those taken after first dosing (including medications that started prior to dosing and continued after).

7.3.10 Exposure to the Investigational Medicinal Product

The actual doses received by subjects will be displayed in a listing.

7.3.11 Pharmacokinetic Concentrations and Variables

The analysis of the PK data will be based on the PK population.

Concentrations below the Lower limit of quantification (LLOQ) will be indicated by 'Below the lower limit of quantification' (BLQ) in the listings.

Pharmacokinetic concentration data will be listed by subject including actual sampling times relative to dosing. Plasma concentrations of metoprolol will be summarized by cohort, treatment and time point, the pre-dose concentration will be assigned to the treatment which will follow (tesofensine plus metoprolol). Plasma concentrations of tesofensine will be summarized by cohort and day. In addition the tesofensine concentrations on Days 15, 18 and 21 (pre-dose and 8 h post dose) will be summarized by cohort, treatment and time point, in this summary the pre-dose sample will be assigned to the treatment which will follow (tesofensine plus metoprolol). The following descriptive statistics will be presented for plasma concentrations obtained at each nominal time point: n, n below LLOQ (with %) arithmetic mean, SD, CV%, geometric mean, geometric CV% (calculated as: gCV% =SQRT(e^{s^2}-1)*100; where s is the standard deviation of the log-transformed values), median, minimum and maximum values.

Pharmacokinetic parameters will be listed by subject for both tesofensine and metoprolol. Pharmacokinetic parameters of metoprolol will be summarized by cohort and treatment.

Descriptive statistics for calculated PK parameters will include: n, arithmetic mean, SD, gCV%, geometric mean, median, minimum and maximum values. For t_{max}, only median, minimum and maximum values will be presented.

No descriptive statistics will be determined when fewer than three individual PK parameters are available.

Individual plasma concentration versus actual times of metoprolol will be plotted by cohort and treatment on linear and semi-logarithmic scale. Mean plasma concentrations of metoprolol versus nominal times will also be presented in linear and semi-logarithmic scale. Two layouts will be used, all treatments overlaid on the same plot by cohort and all cohorts overlaid by metoprolol dose. Plots for metoprolol will have an approximately 24 hour time axis, from pre-dose to 24 hours post dose.

Individual plasma concentration versus actual times from first dose of tesofensine will be plotted by cohort on linear and semi-logarithmic scale. Mean plasma concentrations of tesofensine vs nominal times will also be presented in linear and semi-logarithmic scale. Plots for tesofensine will have a 10 day time axis, Day 13 till Day 23, dosing of tesofensine will be shown in plot annotations.

7.3.11.1 Handling of Values Below the Limit of Quantification (BLQ) in Concentration Summaries and Listings

Handling of values below the limit of quantification (BLQ) in listings and for the calculation of descriptive statistics at each time point:

All BLQ concentrations or missing concentrations will be labeled as such in the concentration data listings. Missing samples will be reported as no sample ("NS") and excluded from analysis. Values that are BLQ will be substituted with BLQ/2 for the calculation of descriptive statistics of concentration by time point.

Graphical presentation:

Any BLQ values prior to the first quantifiable concentration will be plotted at zero for individual linear/linear graphs and excluded from log/linear graphs.

For graphs of arithmetic and geometric means concentrations will follow the rules of summary statistics.

7.3.12 Pharmacodynamic Variables

The analysis of the PD data will be based on the PD population.

The baseline for PD parameters will be the Day -1 measurement. A second baseline will be derived using a matched Day; Days 14, 17, 20 and -1 are baseline for Day 15, 18, 21 and 23 respectively.

In this section the name 'Tesofensine days' will be used to indicate the days on which only tesofensine was dosed, these are days 14, 17, 20 and post dose-administration on day 23. These tesofensine days are typically summarized by treatment and day and in addition all days are combined, giving summaries where each subject by protocol contributes four measurements.

Some PD variables (Pulse, SBP, DBP, HR) are also safety variables and will be listed as part of the safety assessments. They will be listed again, together with the Holter derived PD parameters (M24HR, maximum and minimum HR24, mean HR from designated period and mean HR in quiet hour) by cohort, subject and Day, and will include Day, absolute values, changes from baseline and change from matched day as applicable.

Times of HRmin and HRmax will be listed by cohort, subject and Day, including time relative to dosing. For Baseline (Day -1), a fictive datetime of dosing will be used, this will be 24 hours before Dosing on Day 1.

PD variable summaries of all PD parameters except times of HRmin and HRmax will contain n, mean, SD, median, minimum, maximum. Times of HRmin and HRmax will be summarized categorically.

A pairwise summary will show PD results by tesofensine dose (0.25, 0.50 and 0.75 mg) including change from Baseline and change from Matched day (metoprolol dose).

- Baseline (Day -1) and Day 23.
- Pairs of Day 14 with 15, 17 with 18 and 20 with 21 by metoprolol dose.
    - Metoprolol 25 mg, Day before Metoprolol 25 mg
    - Metoprolol 50 mg, Day before Metoprolol 50 mg
    - Metoprolol 100 mg, Day before Metoprolol 100 mg

The M24HR data will be shown in two box plots. One plot with all days showing observed data, a second box plot showing change from Matched Day.

These same pairs of days will be used to create a pairwise frequency count of relative times of HRmin and HRmax per hour after dosing.

The absolute values and the change from Baseline in M24HR on Tesofensine days will be summarized descriptively by tesofensine dose and Day. This summary will include a summary of Day -1 (providing summary of baseline data) and a mean combining Day 14, 17, 20 and 23.

7.3.12.1 Analysis to check M24HR assumptions on Tesofensine days

This concerns three analyses, comparing days, carry over effect of metoprolol and equality of M24HR prior to metoprolol. Observation of significant effects in these analyses will not be taken into consideration in the selection of mitigating dose.

Values of M24HR on Days 14, 17, 20 and 23 will be compared by means of analysis of variance (ANOVA) with random subjects nested under sequence by cohort. Means, differences between means and CI of differences will be presented. In this analysis there will be no period effect and treatment effect (such as metoprolol dose) other than the fixed days. The following code will be used as basis for the analysis.

```
PROC MIXED;
        BY cohort;
        CLASS sequence day subject;
        MODEL M24HR=day sequence;
        RANDOM subject (sequence);
        LSMEANS day / CL alpha=0.05 diff;
        ODS OUTPUT LSMEANS=ls_means;
QUIT;
```

Carry over of metoprolol will be analyzed using data of Days 17, 20 and 23. In this model the M24HR will be compared using the metoprolol dose of previous days (Day 15, 18 and 21) respectively. The model will be an analysis of variance (ANOVA) with random subjects nested under sequence by cohort. Means, differences between means and CI of differences will be presented. The following code will be used as basis for the analysis.

```
PROC MIXED;
        BY cohort;
        CLASS sequence day subject treatment;
        MODEL M24HR=treatment day sequence;
        RANDOM subject (sequence);
        LSMEANS treatment / CL alpha=0.05 diff;
        ODS OUTPUT LSMEANS=ls_means;
QUIT;
```

Differences in M24HR before dosing will be analysis using data of Days 14, 17 and 20. In this model the M24HR will be compared using the metoprolol dose of the subsequent days (15, 18 and 21). The

model will be an analysis of variance (ANOVA) with random subjects nested under sequence by cohort. Means, differences between means and CI of differences will be presented. The following code will be used as basis for the analysis.

```
PROC MIXED;
        BY cohort;
        CLASS sequence day subject treatment;
        MODEL m24hr=treatment day sequence;
        RANDOM subject (sequence);
        LSMEANS treatment / CL alpha=0.05 diff;
        ODS OUTPUT LSMEANS=ls_means;
QUIT;
```

7.3.12.2 Statistical Analysis for mitigating dose

Mitigating dose is estimated using a linear regression modelling mean change from pre-tesofensine baseline (Day -1) of M24HR as a function of the log metoprolol dose. Based on the regression equation a dose for no effect will be estimated. The final estimate and CI will be obtained using bootstrapping.

As a first step, change from pre-tesofensine baseline (Day -1) of M24HR on Days 15, 18 and 21 will be compared by means of cross-over ANOVA with random subjects nested under sequence and fixed Day (Day 15, 18 and 21 will play the role of period in a conventional cross-over), treatments and baseline by cohort. Means, differences between means and CI of differences will be presented. In this analysis the metoprolol dose will be distinguishing between treatments.

```
PROC MIXED;
        BY cohort;
        CLASS treatment day sequence subject;
        MODEL cfb_m24hr=treatment sequence day baseline;
        RANDOM subject(sequence);
        LSMEANS treatment / CL ALPHA=0.05 DIFF;
        ODS OUTPUT LSMEANS=ls_means;
QUIT;
```

In the final analysis, bootstrapping will be used. The bootstrap samples will be obtained by random selection of subjects in each bootstrap sample. At least 5000 samples will be taken, though more may be used by judgement of the statistician, important consideration for increasing the number of samples will be shape and smoothness of the distribution curve of the final parameter. The ANOVA model above will be used to obtain mean levels for each metoprolol dose. A straight line linear regression will be used to fit the means to log metoprolol dose and the mitigating dose for the sample computed. Mitigating dose will be estimated as mean of bootstrap sample estimates. Confidence intervals will be determined using

the percentiles of the bootstrap estimates. A density plot of the bootstrap estimates for the mitigating dose and for the estimated slopes will be provided. The following code will be used as basis for the estimation.

```
PROC MIXED;
        BY cohort bootstrap_sample;
        CLASS treatment day sequence subject;
        MODEL cfb_m24hr=treatment sequence day baseline;
        RANDOM subject(sequence);
        LSMEANS treatment / CL ALPHA=0.05;
        ODS OUTPUT LSMEANS=ls_means;
QUIT;

PROC REG OUTTEST=outtest;
        BY cohort bootstrap_sample;
        MODEL ls_means=log_metoprolol_dose;
RUN;

DATA result;
        SET outtest;
        log_est=-intercept/slope_of_log_metoprolol_dose;
        est=EXP(log_est);
RUN;
```

Bootstrap samples will be removed before summary, for either of the following reasons:

1. Intercept and/or slope could not be estimated
2. Slope is positive (medically implausible)

In case more than 10% of samples are excluded for any reason, the results will not be displayed. If the percentage of samples discarded due to the first reason is too high (e.g. brings the total to over 10%) in the judgment of the statistician, the statistician may decide to simplify the model regarding regression or covariance structure.

The final model will be displayed in a footnote in all relevant outputs. The summary tables will display number and percentage of removed samples by reason for removal, as well as the bootstrap estimates with CIs.

If the sample size is small, it is expected that the bootstrap samples can include slopes very close to zero. This would lead to implausibly high estimates for the mitigating dose. To evaluate the potential influence the following will be part of the raw statistical output of the model: histogram of the mitigating dose over the bootstrap samples and comparison of median estimate vs mean estimate.

In case the linear model linking Mean M24HR to log dose is clearly not satisfactory, an alternative strategy may be employed, for instance, by interpolation between 2 adjacent doses. The need and approaches for such analysis will be discussed after the initial analysis is finished and will be described in an addendum if alternative analysis will be performed.

7.3.12.3 Sensitivity Analysis

For each Cohort, a sensitivity analysis will be calculated using Fieller CI (Hirschberg and Lye, Beyene and Moineddin). For this calculation a linear relation between CfB M24HR and metoprolol dose will be calculated directly in PROC MIXED. Based on the PROC MIXED the CI are calculated using the following formulas:

$$\hat{\theta} + \left(\frac{k}{1-k}\right)\left(\hat{\theta} + \frac{V_{12}}{V_{22}}\right) \pm \frac{z_{\alpha/2}}{\hat{\beta}(1-k)} \sqrt{\left\{V_{11} + 2\hat{\theta}V_{12} + \hat{\theta}^2 V_{22} - k\left(V_{11} - \frac{V_{12}^2}{V_{22}}\right)\right\}}$$

Using:

$$k = z_{\alpha/2}^2 \frac{V_{22}}{\hat{\beta}^2}$$

$$\hat{\theta} = \hat{\alpha}/\hat{\beta}$$

With $\hat{\alpha}$ and $\hat{\beta}$ the regression estimates and V their Variance-Covariance matrix

The following SAS code will be used as basis for the PROC MIXED calculation:

```
PROC MIXED;
        CLASS subject day sequence;
        MODEL cfb_m24hr = log_dose  sequence   day  / covb;
        RANDOM subject(sequence);
        ESTIMATE 'intercept' intercept 1 log_dose 0;
        ESTIMATE 'slope' intercept 0 log_dose 1;
        ODS OUTPUT Estimates=estimates covb=covb;
RUN;
```

7.3.13 Pharmacokinetic/Pharmacodynamic Analysis

7.3.13.1 Analysis of PD against metoprolol in plasma

In this analysis, a time matched change from baseline for the HR will be employed. In particular this means that baseline will be the HR on time points corresponding to PK measurement points on Day -1. (pre-dose and 2, 4, 8, 12 and 24 hour post dose on Days 15, 18, 21 with 24, 22, 20, 12 and 0 hours

pre-dose as baseline). These are triplicate measurements; in this analysis the mean of the triplicates will be used, the original three measurements of the triplicates will not be displayed in this section, they will be part of the safety listings. If one or two of the triplicate measurements are missing, then the remaining data will be used to obtain the mean. If all three are missing then the observation will be missing. If all three are missing on day -1 for a subject, then the change from baseline for the complete time point will be missing for the subject.

A listing will be provided showing mean of triplicate HR, time matched change from baseline HR, and metoprolol plasma concentration by cohort and nominal time point. This listing will include Day -1 (where only HR will be listed). Plots will be provided showing time matched change from baseline against metoprolol plasma concentration by cohort.

7.3.13.2 Analysis of PD against metoprolol PK parameters

This analysis is the equivalent of the analysis in Section 7.3.12.2. However rather than log metoprolol dose the log PK parameters will be used. Hence an additional step is introduced, calculation of mean log PK parameters. For this a cross over mixed model using fixed treatment day and sequence with random subjects nested under sequence will be used. The following code will be used as basis for the analysis.

```
PROC MIXED;
        BY cohort bootstrap_sample;
        CLASS treatment day sequence subject;
        MODEL cfb_m24hr=treatment sequence day baseline;
        RANDOM subject(sequence);
        LSMEANS treatment / CL ALPHA=0.05;
        ODS OUTPUT LSMEANS=ls_means_m24hr;
QUIT;

PROC MIXED;
        BY cohort bootstrap_sample;
        CLASS treatment day sequence subject;
        MODEL log_pkparam=treatment sequence day;
        RANDOM subject(sequence);
        LSMEANS treatment / CL ALPHA=0.05;
        ODS OUTPUT LSMEANS=ls_means_log_pkparam;
QUIT;

PROC REG OUTTEST=outtest;
        BY cohort bootstrap_sample;
        MODEL ls_means_m24hr=ls_means_log_pkparam;
RUN;
```

```
DATA result;
        SET outtest;
        log_est=-intercept/slope_of_log_pkparam;
        est=EXP(log_est);
RUN;
```

A straight line regression will be used to fit change from baseline M24HR against log PK parameters (AUC_{0-24} and C_{max}) of metoprolol for each tesofensine dose. Based on the regression equation a parameter value for no effect will be estimated. Confidence intervals of the no effect parameter value will be determined using a bootstrap method, again using at least 5000 bootstrap samples. The bootstrap samples will be obtained by random selection of subjects. Plots of change from M24HR as a function of log PK parameter will be displayed by cohort and will include the regression line.

7.3.13.3 Population modelling

A population PK-PD model may be developed if deemed appropriate on the data from this study with the final objective of describing relative contribution to the final effect of plasma exposure to tesofensine and metoprolol, simulating optimal dose regimen for multiple administration and evaluate effect of covariates (e.g., polymorphism of PK, body weight, gender) on the overall effect.

Details of the analysis will be included in a separate Data Analysis plan and outcome of the modelling will be reported separately, if population PK-PD modeling is conducted.

7.3.14 Safety Analysis

The analysis of the safety variables will be based on the safety population.

7.3.14.1 Adverse Events

The following listings will be produced:

- All pre-treatment AEs and TEAEs (by subject).
- Withdrawals due to AEs (if applicable).
- Serious Adverse Events (SAEs) (if applicable).
- Serious Adverse Events Leading to Death (if applicable).

The following information will be included in the listings: AE number, reported term, System Organ Class (SOC), Preferred Term (PT), start and end date/time, intensity, causality, action taken, outcome, classified as serious and treatment emergence.

Numbers of TEAEs will be summarized by treatment, SOC, PT, and also by treatment, SOC, PT and severity and also by treatment, SOC, PT and causality to treatment. In addition serious TEAES will be summarized by treatment, SOC and PT.

For all summaries of TEAEs the treatments of Section 7.3.1 will be used. In addition the summaries for the following combined treatments will be created:

- Cohort 1
- Cohort 2
- Cohort 3
- Tesofensine without Metoprolol (all doses)
- Metoprolol 25 mg (Combining all Tesofensine doses)
- Metoprolol 50 mg (Combining all Tesofensine doses)
- Metoprolol 100 mg (Combining all Tesofensine dose)
- Tesofensine and Metoprolol (combining all doses)
- All TEAE combined

7.3.14.2 Clinical Safety Laboratory Tests (hematology, clinical chemistry and urinalysis)

Laboratory values (hematology, clinical chemistry and urinalysis) will be listed by subject and study time point including changes from baseline (with the exception of urinalysis). The baseline for the laboratory values will be the results obtained on Day -2.

All values outside the clinical reference ranges will be flagged in the data listings. The abnormal values will be flagged with 'L' for values below the lower limit of the clinical reference range and 'H' for values above the upper limit of the clinical reference range and included in the listings. The Investigator will assess whether the values outside the clinical reference range are clinically significant and these will be reported as abnormal NCS or abnormal CS. Clinically significant laboratory values will be recorded by the Investigator as AEs.

Descriptive statistics (for non-categorical data including hematology and clinical chemistry) will be presented by Cohort, visit and treatment for both individual values (N, mean, SD, median, minimum, maximum) and changes from baseline.

Shift tables for hematology and clinical chemistry will be presented by Cohort, visit and treatment.

7.3.14.3 Vital Signs

Vital signs data will be listed by subject including changes from baseline. The baseline for the vital signs measurements will be the pre-dose measurement on Day 1. Baseline for Body weight and waist circumference will be Day -2.

Triplicate measurements of vital signs will be listed with both the original measurements and the mean for the time point. For triplicate measurements the mean within a time point will be used for summaries, baseline and change from baseline as applicable.

Descriptive statistics (n, mean, SD, median, minimum, maximum) for absolute values and changes from baseline will be presented by treatment and time point or treatment, visit and time point for days where only tesofensine is dosed.

7.3.14.4 Twelve-Lead Electrocardiogram

All ECG parameters obtained from the ECG measurement will be listed by subject for each treatment and time point including changes from baseline. The baseline for the ECG measurements will be pre-dose measurement obtained on Day 1.

Descriptive statistics (n, mean, SD, median, minimum, maximum) for absolute values and changes from baseline will be presented by treatment and time point.

7.3.14.5 Physical Examination

The results of the physical examination will be listed by subject and time-point.

7.3.14.6 Patient Health Questionnaire-9 and (PHQ-9) and Generalized Anxiety Disorder Assessment (GAD-7)

Results of the questionnaires will be listed by subject. Sum scores of PHQ-9 and GAD-7 will summarized by cohort and overall using the safety population.

7.3.14.7 Additional assessments based on BfArM's request

Additional assessments will be listed, including further actions recommended and performed and references to AEs and concomitant medications added.

8. REFERENCES

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2. Pharsight® WinNonlin Professional Software Version 6.3. http://www.pharsight.com
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4. Hirschberg J, Lye J. A Geometric Comparison of the Delta and Fieller Confidence Intervals. The American Statistician, August 2010, 234-241.
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9. SCHEDULE OF ASSESSMENTS

9.1 Schedule of Assessments for Cohort 1 and Cohort 2

Evaluation	Screening	Base-line		Treatment Period																Safety FU Phone Calls	
Day	**-28 to -3**	**-2**	**-1[a]**	**1**	**2**	**3**	**7**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**30**	**50**
Admission		X						X													
Discharge						X													X		
In-house stay[b]		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		
Ambulatory visits	X						X														
Phone call																				X	X
Informed consent	X																				
Medical history	X																				
Demographics	X																				
Inclusion/exclusion criteria	X																				
Physical examination	X																		X		
Body weight	X	X							X			X			X				X		
Waist circumference	X	X							X			X			X				X		
Height, calculation of BMI	X																				
Blood sampling for CYP2D6	X																				
Viral serology	X																				
Clinical laboratory tests (clinical chemistry, hematology and urinalysis) in fasted state	X	X					X												X		
HBA1c	X																				
Serum pregnancy test[m]	X																		X		

Evaluation	Screening	Base-line		Treatment Period																Safety FU Phone Calls	
Day	**-28 to -3**	**-2**	**-1[a]**	**1**	**2**	**3**	**7**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**30**	**50**
Urine pregnancy test[m]		X						X													X[n]
Psychodiagnostic self-rating instruments (PHQ-9 and GAD-7)	X																				
Urine drug screen, cotinine and alcohol test	X	X						X													
24-hours Holter ECG[c]			X[a]						X	X		X	X		X	X		X			
12-lead ECG[d,f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Supine blood pressure and pulse[e,f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Body temperature[g]	X	X	X						X	X		X	X		X	X		X			
A designated period from 3 to 16 hours post-dose and a quiet hour from 12 to 13 hours post-dose[f]			X						X	X		X	X		X	X		X			
Randomization[h]				X																	
Dosing of tesofensine[i]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Dosing of metoprolol[j]										X			X			X					
Blood sampling for PK[k]								X	X	X			X			X		X			
Dispense of tesofensine container						X															
Subject returns tesofensine container to the clinical unit								X													
Dispense of the diary and instructions[l]					X																
Collection of the diary								X													
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Evaluation	Screening	Base-line		Treatment Period																Safety FU Phone Calls	
Day	**-28 to -3**	**-2**	**-1[a]**	**1**	**2**	**3**	**7**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**30**	**50**
AE monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

AE = adverse event; BMI = Body Mass Index; ECG = electrocardiogram; FU = Follow-up; PK = pharmacokinetics

a The 24-hours ECG recording at pre-tesofensine baseline may be repeated, if less recording time than 22 hours for comparison of the data is available for evaluation due to technical issues. Then dosing and all subsequent assessments will be postponed by 1 day.

b Subjects will be admitted in the morning of Day -2 and will leave the study center in the morning of Day 3 after dosing of the last loading dose and after completion of all study-related assessments.
Subject will take tesofensine at home once daily from Day 4 to Day 6 and from Day 8 to Day 12.
On Day 7, subject will come to the study center for an ambulatory visit (including dosing).
Subjects will be admitted in the morning of Day 13 and will leave the study center on Day 24 after completion of all study-related assessments.

c 2-lead Holter-ECG recordings will start in the morning before dosing (or at corresponding time at pre-tesofensine baseline) and will end 24 hours after respective dose.

d On Days -1, 14, 15, 17, 18, 20, 21 and Day 23: Triplicate measurement (i.e., 3 recordings of 10 seconds each, at least 60 seconds apart) within 10 minutes before each PK sampling and at corresponding time points (pre-dose and at 2, 4, 8, 12 and 24 hours post-dose).
On other days and at Screening, also in triplicates: once daily in the morning (pre-dose on dosing days).

e On Days -1, 14, 15, 17, 18, 20, 21 and Day 23: Triplicate measurement within 10 minutes before each PK sampling and at corresponding time points (pre-dose and at 2, 4, 8, 12 and 24 hours post-dose).
On other days and at Screening, also in triplicates: once daily in the morning (pre-dose on dosing days).

f Subjects will have to rest during the quiet hour and for at least 10 minutes before 12-lead ECG recording and blood pressure and pulse measurements, i.e., each subject will be asked to find a personally comfortable supine position with the head and whole body firmly supported. The subjects will not be permitted to watch television or to listen to radio or music player. They will not be permitted to read, to sleep or converse, and will be kept free from any external disturbance (e.g., personal media). No electric equipment including mobile phones may be switched on in the room.

g Once daily, on in-house days in the morning.

h Randomization will be performed pre-dose on Day 1.

i Dosing of tesofensine will start in the morning of Day 1 and will be continued until the morning of Day 23, when the last dose of tesofensine will be given; subjects will be randomly assigned to any of the 3 tesofensine dose cohorts (Cohort 1, Cohort 2, Cohort 3).

Cohort 1: 1.0 mg once daily for the first 3 days (loading dose), then 0.25 mg once daily.
Cohort 2: 2.0 mg once daily for the first 3 days (loading dose), then 0.50 mg once daily.

j Dosing of extended release metoprolol will be performed in the morning of Day 15, Day 18 and Day 21 within 30 minutes after start of a breakfast.
Administration of single doses of 25 mg, 50 mg and 100 mg in random order.

k Blood sampling for metoprolol: at pre-dose and at 2, 4, 8, 12 and 24 hours post-dose on Day 15, Day 18 and Day 21.
Blood sampling for tesofensine: at pre-dose on Day 13, Day 14 and Day 15 to confirm steady state, at pre-dose on Day 18, Day 21 and Day 23 and at 8 hours post-dose on Day 15, Day 18 and Day 21.

l On outpatient days, date and time of dosing, adverse events (if any) and use of concomitant medication (if any) will be documented in a diary.

m Pregnancy tests will be performed whenever deemed necessary.

n The pregnancy test on Day 50 will be performed at home and the result will be communicated during the safety FU phone call.

9.2 Schedule of Assessments for Cohort 3

Evaluation	Scree-ning	Base-line		Treatment Period																		Safety FU Phone Calls	
Day	**-28 to -3**	**-2**	**-1[a]**	**1**	**2**	**3**	**4**	**5**	**7**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**30**	**50**
Admission		X								X													
Discharge						X															X		
In-house stay[b]		X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X		
Ambulatory visits	X						X	X	X														
Phone call																						X	X
Informed consent	X																						
Medical history	X																						
Demographics	X																						
Inclusion/exclusion criteria	X																						
Physical examination	X																				X		
Body weight	X	X									X			X			X				X		
Waist circumference	X	X									X			X			X				X		
Height, calculation of BMI	X																						
Blood sampling for CYP2D6	X																						
Viral serology	X																						
HBA1c	X																						
Clinical laboratory tests (clinical chemistry, hematology and urinalysis) in fasted state	X	X							X												X		
Serum pregnancy test	X																				X		
Urine pregnancy test		X								X													X[n]
Psychodiagnostic self-rating instruments (PHQ-9 and GAD-7)	X																						

Evaluation	Scree-ning	Base-line		Treatment Period																		Safety FU Phone Calls	
Day	-28 to -3	-2	-1[a]	1	2	3	4	5	7	13	14	15	16	17	18	19	20	21	22	23	24	30	50
Urine drug screen, cotinine and alcohol test	X	X								X													
24-hours Holter ECG[c]			X[a]								X	X		X	X		X	X		X			
12-lead ECG[d,f]	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X		
Supine blood pressure and pulse[e,f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Body temperature[g]	X	X	X								X	X		X	X		X	X		X			
A designated period from 3 to 16 hours post-dose and a quiet hour from 12 to 13 hours post-dose[f]			X								X	X		X	X		X	X		X			
Randomization[h]				X																			
Dosing of tesofensine[i]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Dosing of metoprolol[j]												X			X			X					
Blood sampling for PK[k]										X	X	X			X			X		X			
Dispense of tesofensine containers								X															
Subject returns tesofensine containers to the clinical unit										X													
Dispense of the diary and instructions[l]					X																		
Collection of the diary										X													
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

AE = adverse event; BMI = Body Mass Index; ECG = electrocardiogram; FU = Follow-up; PK = pharmacokinetics

a The 24-hours ECG recording at pre-tesofensine baseline may be repeated, if less recording time than 22 hours for comparison of the data is available for evaluation due to technical issues. Then dosing and all subsequent assessments will be postponed by 1 day.

b Subjects will be admitted in the morning of Day -2 and will leave the study center in the morning of Day 3 after dosing of the last loading dose and after completion of all study-related assessments.

Subjects will come to the study center for ambulatory visits and dosing of tesofensine on Days 4 and 5.
Subjects will take tesofensine at home once daily on Day 6 and from Day 8 to Day 12.
On Day 7, subject will come to the study center for an ambulatory visit (including dosing).
Subjects will be admitted in the morning of Day 13 and will leave the study center on Day 24 after completion of all study-related assessments.

c 2-lead Holter-ECG recordings will start in the morning before dosing (or at corresponding time at pre-tesofensine baseline) and will end 24 hours after respective dose.

d On Days -1, 14, 15, 17, 18, 20, 21 and Day 23: Triplicate measurement (i.e., 3 recordings of 10 seconds each, at least 60 seconds apart) within 10 minutes before each PK sampling and at corresponding time points (pre-dose and at 2, 4, 8, 12 and 24 hours post-dose).
On other days and at Screening, also in triplicates: once daily in the morning (pre-dose on dosing days).

e On Days -1, 14, 15, 17, 18, 20, 21 and Day 23: Triplicate measurement within 10 minutes before each PK sampling and at corresponding time points (pre-dose and at 2, 4, 8, 12 and 24 hours post-dose).
On other days and at Screening, also in triplicates: once daily in the morning (pre-dose on dosing days).

f Subjects will have to rest during the quiet hour and for at least 10 minutes before 12-lead ECG recording and blood pressure and pulse measurements, i.e., each subject will be asked to find a personally comfortable supine position with the head and whole body firmly supported. The subjects will not be permitted to watch television or to listen to radio or music player. They will not be permitted to read, to sleep or converse, and will be kept free from any external disturbance (e.g., personal media). No electric equipment including mobile phones may be switched on in the room.

g Once daily, on in-house days in the morning.

h Randomization will be performed pre-dose on Day 1.

i Dosing of tesofensine will start in the morning of Day 1 and will be continued until the morning of Day 23, when the last dose of tesofensine will be given; subjects will be randomly assigned to any of the 3 tesofensine dose cohorts (Cohort 1, Cohort 2, Cohort 3).
Cohort 3: 2.0 mg once daily for the first 5 days (loading dose), then 0.75 mg once daily.

j Dosing of extended release metoprolol will be performed in the morning of Day 15, Day 18 and Day 21 within 30 minutes after start of a breakfast.
Administration of single doses of 25 mg, 50 mg and 100 mg in random order.

k Blood sampling for metoprolol: at pre-dose and at 2, 4, 8, 12 and 24 hours post-dose on Day 15, Day 18 and Day 21.
Blood sampling for tesofensine: at pre-dose on Day 13, Day 14 and Day 15 to confirm steady state, at pre-dose on Day 18, Day 21 and Day 23 and at 8 hours post-dose on Day 15, Day 18 and Day 21.

l On outpatient days, date and time of dosing, adverse events (if any) and use of concomitant medication (if any) will be documented in a diary.

m Pregnancy tests will be performed whenever deemed necessary.

n The pregnancy test on Day 50 will be performed at home and the result will be communicated during the safety FU phone call.

10. TABLES AND LISTINGS TO BE INCLUDED IN SECTION 14 OF THE CLINICAL STUDY REPORT

Subject Disposition

Table 14.1.1 Subject Disposition (Safety Population)

Table 14.1.2 Subject Disposition by Cohort (Safety Population)

Baseline and Demographic Data

Table 14.1.3 Subject Demographics by Cohort (Safety Population)

Pharmacokinetic and pharmacodynamic Data

Table 14.2.1 Summary of Plasma Concentrations of Tesofensine versus Nominal Sampling Times by Tesofensine Dose (PK Population)

Table 14.2.2 Summary of Plasma Concentrations of Metoprolol versus Nominal Sampling Times by Metoprolol Dose and Cohort (PK Population)

Table 14.2.3 Summary of Pharmacokinetic Parameters of Tesofensine by Tesofensine Dose and Visit (PK Population)

Table 14.2.4 Summary of Pharmacokinetic Parameters of Metoprolol by Metoprolol Dose and Cohort (PK Population)

Table 14.2.5 Summary of Pharmacodynamic Parameters (PD Population)

Table 14.2.6 Frequency of Times of Minimum and Maximum Heart Rate by Hour (PD Population)

Table 14.2.7 Summary of M24HR by Metoprolol Dose (PD Population)

Table 14.2.8 Comparison of M24HR on Tesofensine Days by Day (PD Population)

11. FIGURES

Figure 14.2.1 Subject Profiles for Metoprolol Plasma Concentration Time Data (Linear Scale) (Safety Population)

Figure 14.2.2 Subject Profiles for Metoprolol Plasma Concentration Time Data (Semi-logarithmic Scale) (Safety Population)

Figure 14.2.3 Subject Profiles for Tesofensine Plasma Concentration Time Data (Linear Scale) (Safety Population)

Figure 14.2.4 Subject Profiles for Tesofensine Plasma Concentration Time Data (Semi-logarithmic Scale) (Safety Population)

Figure 14.2.5 Mean (± Standard Deviation) Profiles for Metoprolol Plasma Concentration Time Data (Linear Scale) (PK Population)

Figure 14.2.6 Mean Profiles for Metoprolol Plasma Concentration Time Data (Semi-logarithmic Scale) (PK Population)

Figure 14.2.7 Mean (± Standard Deviation) Profiles for Tesofensine Plasma Concentration Time Data (Linear Scale) (PK Population)

Figure 14.2.8 Mean Profiles for Tesofensine Plasma Concentration Time Data (Semi-logarithmic Scale) (PK Population)

Figure 14.2.9 Individual M24HR vs Treatment (PD Population)

Figure 14.2.10 Combined Individual M24HR vs Treatment by Tesofensine Dose (PD Population)

Figure 14.2.11 Box Plot of M24HR vs. Metoprolol dose by Tesofensine Dose (PD Population)

Figure 14.2.12 Box Plot of Change from Matched Day M24HR vs. Metoprolol Dose by Tesofensine Dose (PD Population)

Figure 14.2.13 Display of Bootstrap Results by Tesofensine Dose (PD Population)

Figure 14.2.14 Combined Display of Bootstrap Results (PD Population)

Figure 14.2.15 Density Plot for Estimated Metoprolol Dose to Compensate M24HR by Tesofensine Dose (PD Population)

Figure 14.2.16 Time Matched Change from Baseline HR against Metoprolol Plasma Concentration (PK Population)

Figure 14.2.17 Density Plot for Estimated PK Parameter to Compensate M24HR by Tesofensine Dose (PD Population)

12. LISTINGS TO BE INCLUDED IN SECTION 16 OF THE CLINICAL STUDY REPORT

Subject Disposition

Listing 16.2.1.1 Withdrawals from the Study (Safety Population)

Listing 16.2.1.2 Informed Consent (Safety Population)

Listing 16.2.1.3 Study Visits (Safety Population)

Listing 16.2.1.4 Failed Inclusion and Exclusion Criteria (Safety Population)

Listing 16.2.2 Protocol Deviations (Safety Population)

Listing 16.2.3 Assignment to Analysis Populations (Safety Population)

Baseline and Demographic Data

Listing 16.2.4.1 Subject Demographics (Safety Population)

Listing 16.2.4.2 Medical History (Safety Population)

Listing 16.2.4.3 Subject Habits (Safety Population)

Concomitant Medication

Listing 16.2.4.4 Prior and Concomitant Medications (Safety Population)

Exposure

Listing 16.2.5.1 Exposure to Study Drug (Safety Population)

Listing 16.2.5.2 Individual Blood Sampling Times and Concentrations (Safety Population)

Pharmacokinetic Data

Listing 16.2.6.1 Pharmacokinetic Parameters of Metoprolol (PK Population)

Listing 16.2.6.2 Pharmacokinetic Parameters of Tesofensine (PK Population)

Pharmacodynamic Data

Listing 16.2.6.3 Pharmacodynamic Variables and Change from Baseline (PD Population)

Listing 16.2.6.4 Times of HRmin and HRmax including times relative to dosing (PD Population)

Listing 16.2.6.5 Heart Rate with time matched change from baseline (PD Population)

Safety Variables

Listing 16.2.7 Adverse Events (Safety Population)

Listing 16.2.8.1 Clinical Chemistry (Safety Population)

Listing 16.2.8.2 Hematology (Safety Population)

Listing 16.2.8.3 Urinalysis (Safety Population)

Listing 16.2.8.4 Serology (Safety Population)

Listing 16.2.8.5 Drugs of Abuse (Safety Population)

Listing 16.2.8.6 Other Clinical Laboratory (Safety Population)

Listing 16.2.9.1 Vital Signs (Safety Population)

Listing 16.2.9.2 Electrocardiogram Parameters (Safety Population)

Listing 16.2.9.3 Physical Examination (Safety Population)

Listing 16.2.9.4 Patient Health Questionnaire-9 (PHQ-9) and Generalized Anxiety Disorder Assessment (GAD-7) (Safety Population)

Listing 16.2.9.5 Safety Assessments based on BfArM's Request (Safety Population)

13. DOCUMENTATION OF STATISTICAL METHODS

Appendix 16.1.9.2.1 Comparison of M24HR on Tesofensine Days by Day (PD Population)

Appendix 16.1.9.2.2 Comparison of M24HR of Days 14, 17 and 20 by subsequent Metoprolol dose (Pharmacodynamic Population)

Appendix 16.1.9.2.3 Comparison of M24HR of Days 17, 20 and 23 by preceding Metoprolol dose (Pharmacodynamic Population)

Appendix 16.1.9.2.4 Comparison of Change from Baseline M24HR by Metoprolol dose (PD Population)

Appendix 16.1.9.2.5 WinNonLin Outputs