STUDY PROTOCOL

Drug Cocktail Interaction Study to Investigate the Effect of St. John's Wort dry Extract Ze 117 on Several Cytochrome P450 Enzymes and on Transporter P-Glycoprotein in Healthy Volunteers.

EudraCT number:	2017-003760-12
Sponsor's study code:	Ze117-1-2017-01
CRO's study code:	N-A-PH1-17-025
Phase:	1
Name of investigational medicinal product(s):	St. John's wort film coated tablets 500 mg
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Version/Date:	final 2.0, 15-JAN-2018

CONFIDENTIALITY STATEMENT

This protocol is the property of Max Zeller Söhne AG. The recipient agrees that no unpublished information contained herein will be published or disclosed without prior written approval of the Sponsor, except that this protocol may be disclosed to the appropriate Independent Ethics Committee or Institutional Review Board and appropriate Authority(ies).

This report was written according to the ICH E6 Guideline: Good Clinical Practice: Consolidated guideline, CPMP/ICH/135/95

SIGNATURE PAGE CRO

The people signing hereby declare that they have read this protocol and agree to its contents. They confirm that the study will be conducted and documented in full accordance with the protocol, standards of World Medical Association Declaration of Helsinki, International Conference on Harmonization and Good Clinical Practice specified herein, and the national drug law.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADR	adverse drug reaction
AE	adverse event
ALP	alkaline phosphatase
AMG	Arzneimittelgesetz (German Drug Law)
AUC	area under the time versus concentration curve
BfArM	Federal Institute for Drugs and Medical Products
BMI	body mass index
CA	competent authority
C_{max}	maximum concentration
CRF	case report form
CRO	Contract Research Organization
CTS	clinical trial supplies
CV	coefficient of variation
CYP	cytochrome P450
ECG	electrocardiogram
EDTA	ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV 1/2	human immunodeficiency virus 1/2
ICD	International Statistical Classification of Diseases and Related Health Problems
ICF	informed consent form
ICH	International conference on harmonization
IEC	independent ethics committee
IMP	investigational medicinal product
λ_z	terminal elimination rate constant
LC-MS/MS	liquid chromatography followed by tandem mass spectrometry
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
pH	grade of acidity
P-gp	P-glycoprotein
QTcB	QTc interval corrected according to the Bazett's formula
PK	pharmacokinetics
SAE	serious adverse event
SAR	serious adverse drug reaction
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2}$	terminal half-life
TEAE	treatment emergent adverse event
t_{max}	time to reach maximum concentration, C_{max}
TSH	thyroid-stimulating hormone
Vz/f	apparent volume of distribution over bioavailability

WHO	World Health Organization

SYNOPSIS

Title of the study: Drug Cocktail Interaction Study to Investigate the Effect of St. John's Wort dry Extract Ze 117 on Several Cytochrome P450 Enzymes and on Transporter P-Glycoprotein in Healthy Volunteers

Study number: Ze117-1-2017-01

Investigator: Michael Lissy

Study center: Nuvisan GmbH, Center for Human Pharmacology Wegenerstrasse 13, 89231 Neu-Ulm, Germany

Objectives:

Primary objective

- To evaluate a possible interaction (induction or inhibition) of St. John's wort dry extract Ze 117 on several cytochrome P450 (CYP) enzymes and the transporter P-glycoprotein (P-gp) by comparing the primary pharmacokinetic parameters of the cocktail substrates.

Secondary objectives

- To evaluate a possible interaction (induction or inhibition) of St. John's wort dry extract Ze 117 on several cytochrome P450 enzymes and the transporter P-gp by comparing the secondary pharmacokinetic parameters of the cocktail substrates and their relevant metabolites.
- To evaluate the safety of Ze 117 alone and in combination with the probe drugs.

Methodology (design of study):

Phase I, open-label, non-randomized, single-sequence study.

Subjects will be hospitalized from Day -1 until discharge in the morning of Day 18. Subjects will receive 500 mg Ze 117 in the morning of Days 8 to Day 17. A cocktail consisting of 7 probe drugs will be given three times as a single dose each (Day 1, Day 8, Day 17):

The compilation of the probe drugs is according to a validated cocktail (Geneva cocktail) and containing the following probes:

- Caffeine (CYP1A2)
- Bupropion (CYP2B6)
- Flurbiprofen (CYP2C9)
- Omeprazole (CYP2C19)
- Dextromethorphan (CYP2D6)
- Midazolam (CYP3A4)
- Fexofenadine (P-gp)

Plasma samples for determination of the probe drugs and their

	metabolites will be taken until 72 hours after each administration of the cocktail.
Number of subjects:	10 ± 1 male and 10 ± 1 female participants are planned to be included in this study.
Diagnosis and main criteria for inclusion:	Healthy adult males or females aged 18 to 55 years, inclusive at the time of screening; body mass index (BMI) of ≥19 and ≤29 kg/m^2, body weight ≤ 90 kg.
Test product, dose and mode of administration:	Ze 117 (Rebalance® 500; St. John's wort dry Extract), 1 tablet will be taken with 240 ml of water for 10 days (Day 8 to Day 17). The cocktail of 7 probe drugs will be given in the morning of Day1, Day 8, Day 17. Compilation of the probe drugs according to the validated Geneva cocktail: • Caffeine, 50 mg, tablet • Bupropion HCl, 75 mg, tablet • Flurbiprofen, 10 mg, oral solution • Omeprazol, 10 mg, capsule • Dextromethorphan, 10 mg, oral solution • Midazolam, 1 mg, oral solution • Fexofenadine, 25 mg, oral suspension The probe drugs will be taken "at once" without test drug Ze 117 (Day 1) or together with the morning dose of test drug Ze 117 (Days 8 and 17) together with the 240 mL of water.
Reference therapy, dose and mode of administration:	Not applicable.
Duration of treatment:	Ze 117 will be administered for 10 days (Day 8 to Day 17), the cocktail will be administered three times (Day 1, Day 8, Day 17)
Criteria for evaluation:	<u>Pharmacokinetic variables:</u> Blood samples (19) for determination of plasma concentrations of the probe drugs and their metabolites will be collected at: • pre-dose, and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after each cocktail administration. The analytes are: • Caffeine and paraxanthine • Bupropion and 4-hydroxybupropion • Flurbiprofen and 4-hydroxyflurbiprofen • Omeprazole and 5-hydroxyomeprazole • Dextromethorphan and dextrorphan

- Midazolam and 1-hydroxymidazolam
- Fexofenadine

The following parameters will be determined for each probe drug and metabolite:

Primary parameters: area under the plasma concentration-time curve up to the last sampling time with a concentration above the limit of quantification (AUC_{0-t}) at Days 1 and 17 for each of the probe drugs.

Secondary parameters: AUC_{0-t} and area under the plasma concentration-time curve extrapolated to infinity (AUC_{0-inf}), maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), terminal elimination rate constant (λ_z), elimination half-life ($t_{1/2}$) on Days 1, 8 and 17 for each of the probe drugs and their metabolites.

Safety variables:

Adverse events, vital signs (blood pressure, pulse rate, body temperature), 12-lead ECG parameters, physical examination, and safety laboratory investigations.

Statistical methods:

Safety:

All safety data will be evaluated descriptively.

Pharmacokinetics:

Blood concentrations and derived PK parameters will be evaluated descriptively.

In order to assess the effect of multiple doses of Ze 117 on AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the probe drugs (and metabolites) one-way ANOVA models on the log transformed (base e) parameters will be performed. The estimated treatment differences will be transformed back to original scale and presented as a ratio between treatments including the corresponding 90% CI. The CIs will be interpreted based on a pre-specified hierarchical hypothesis testing scheme.

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STUDY DESIGN AND SCHEDULE OF ASSESSMENTS

Table 1 Schedule of assessments

Period	**Scree-ning**															**End of trial**	**Follow-up[g]**
Study day	**D -21 to -2**	**D -1**	**D 1**	**D 2**	**D 3-4**	**D 5-6**	**D 7**	**D 8**	**D 9**	**D 10-11**	**D 12-15**	**D 16**	**D 17**	**D 18**	**D 19**	**D -20**	**D 24 ± 2 days**
Hospitalization		X	X	X	X	X	X	X	X	X	X	X	X	X			
Ambulant visits	X														X	X	
Informed consent*	X																
Check of in/exclusion criteria	X	X															
Medical history/demographics	X																
Height/weight/BMI	X																
Physical examination	X	X		X				X	X				X	X		X	
12-lead ECG	X	X		X				X	X				X	X		X	
Vital signs[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
Safety laboratory[b]	X	X		X				X	X				X	X		X	
Pregnancy test (in females)[c]	X	X												X		X	
Serology testing	X																
Urine drug screen, cotinine test and alcohol breath test	X	X															
Blood sample for genotyping			X														
Ze 117 administration[d]								X	X	X	X	X	X				
Probe drug cocktail administration[e]			X					X					X				

Period	**Scree-ning**															**End of trial**	**Follow-up[g]**
Study day	**D -21 to -2**	**D -1**	**D 1**	**D 2**	**D 3-4**	**D 5-6**	**D 7**	**D 8**	**D 9**	**D 10-11**	**D 12-15**	**D 16**	**D 17**	**D 18**	**D 19**	**D -20**	**D 24 ± 2 days**
Pharmacokinetics sampling[f]			X	X	X			X	X	X			X	X	X	X	
Prior medications	X	X															
Adverse events and concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

* Written informed consent must be available prior to any screening activities

BMI = body mass index, ECG = electrocardiogram

a. Vital signs (supine blood pressure and pulse rate, body temperature): on Days 1, 8 and 17: pre-dose, 2, 4, 8 and 24 hours post-dose, on other days once in the morning, pre-dose if applicable

b. Collection of blood and urine samples for clinical chemistry, hematology, coagulation and urinalysis

c. Serum pregnancy test at screening and urine pregnancy test on Day -1, Day 18 and Day 20 (end of trial examination)

d. Administration of 500 mg Ze 117 for 10 days in the morning of Days 8 to 17

e. The probe drugs will be taken alone (Day 1) or together with the morning dose of Ze 117 (Days 8 and 17)

f. PK samples for probe drugs + metabolites: pre-dose, and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after each cocktail administration. It is acceptable if sampling will take place in a time window of ± 3min, for the time points starting 24 hours post dosing in a time window of ± 30 min. Actual times of PK blood sampling will always be documented in the CRF as well as the deviations from the scheduled blood sampling time points.

g. Assessment of wellbeing via telephone

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1. INTRODUCTION

1.1 BACKGROUND

Zeller's St. John's wort dry extract Ze 117 has the indication for the short term treatment of mild depressive disorders according to the HMPC monograph [1] and is approved in several countries, e.g. in Switzerland as Rebalance® 500 [2] with the indication of depressed mood. In the European Union, Ze 117 is approved in Hungaria as Remotiv® extra [3] with the indication of mild depression symptoms and in Germany as "Remotiv® N Filmtabletten" [4] with the indication of mild temporary depression.

1.2 PRE-CLINICAL DATA

The mechanism of action of St. John's wort extracts is still not clarified in detail. However, pharmacodynamic effects are shown in experimental investigations in animals that show an inhibition of noradrenalin, serotonin and dopamine re-uptake into the pre-synaptic neurons. Also, there is in-vitro data on a down-regulation of central beta-adrenoreceptors. [1, 5, 6]

The effect is attributed to the increase of neurotransmitter concentration in the synaptic cleft as well as to the modulating effect of the neurotransmitters at the postsynaptic membrane.

St. John's wort dry extract changes the behavior of animals in several antidepressant models (e.g. forced swimming test) similarly to synthetic antidepressants. Naphthodianthrones (e.g. hypericin, pseudohypericin), phloroglucin derivatives (e.g. hyperforin) and flavonoids contribute to the activity. [7]

1.3 CLINICAL DATA

The clinical evidence of Remotiv® extra [2, 3] was investigated in three randomized controlled trials and one uncontrolled study.

In a two-armed, double-blind, randomized, placebo-controlled multicenter clinical trial, the efficacy and safety of the active ingredient Ze 117 as a treatment of mild and moderate depressive disorders (F32.0 and F32.1 according to ICD-10) was tested using a daily dose of 2 x 250 mg against a placebo in 162 patients. The validated objective and self-assessment criteria and the responder rate shows statistically significant and clinically relevant superiority of Ze 117 to the placebo treatment. [8]

In a further double-armed, double-blind, randomized and controlled multicenter clinical study, the efficacy and safety of Ze 117 (2 x 250 mg/ day) was compared to the selective serotonin reuptake inhibitor fluoxetine (20 mg/ day) in 240 patients suffering from mild to moderate depressive disorders (F32.0 and F32.1 according to ICD-10) over a period of 6 weeks. In this study Ze 117 showed a statistically comparable efficacy with fluoxetine. However, the responder rate in the Ze 117 group was significantly higher than in the fluoxetine group. Also, the tolerability profile of Ze 117 proved to be significantly more favorable than that of fluoxetine. [9]

In another double-armed, randomized, double-blind multicenter study with 324 patients, the efficacy and safety of Ze 117 (2 x 250 mg/ day; n = 157) during treatment of mild to moderate depressive disorders (F32.0/1 and F33.0/1 according to ICD-10) were investigated in comparison with the tricyclic antidepressant imipramine (150 mg/ day). The extract Ze 117 showed a statistically comparable efficacy with imipramine as well as comparable responder rates. Concerning safety, 39% of patients in the Ze 117 group exhibited adverse reactions in contrast to 63% of patients in the imipramine group.[10]

In an open long term study (52 weeks) with 440 patients, the treatment of mild to moderate depression (ICD-10/F32.0/1 und F33.0/1) with St. John's wort dry extract Ze 117 showed good tolerability of the medication and significant improvement of the symptoms. Ze 117 is therefore a safe and efficient treatment option for the long-term application in patients with mild to moderate depressive disorders.[11]

For further details see the summary of product characteristics Remotiv® extra[2].

1.4 RATIONALE

Current data indicate that St. John's wort preparations may induce hepatic cytochrome P450 enzymes and transport proteins. This can result in drug interactions.

Hyperforin (one of the constituents in St John's wort) has been demonstrated to be responsible for many of the observed drug interactions.

There is reasonable evidence to suggest that low-dose hyperforin extracts have no significant effects on cytochrome P450 (CYP) enzymes such as CYP3A4 and transport proteins such as P-glycoprotein (P-gp). [12], [13]

Therefore, due to its low hyperforin content, Ze 117 may have less potential for drug interactions than other St. John's wort preparations.

Since *in-vitro* data are not always predictive of *in-vivo* behavior of the compound tested, a study in healthy human subjects has to be performed. The aim of the study is to investigate the effect of Ze 117 on the interaction potential with relevant P450 (cytochrome P450) enzymes and P-pg transporter.

The study design is standard for DDI studies and is based on the regulatory guidance of the Food and Drug Administration (FDA)[14] and of the European Medicines Agency (EMA).[15]

A cocktail approach involving the administration of multiple cytochrome P450 (CYP)- or P-glycoprotein (P-gp)-specific probe drugs is used to simultaneously assess the activities of these enzymes and the transporter P-gp. Many phenotyping cocktails have been developed and used over the past years. For this study the compilation of the probe drugs is selected according to a validated cocktail (Geneva cocktail).[16], [17] Selected probe drugs are simultaneously administered in low doses such as caffeine (CYP1A2), bupropion (CYP2B6), flurbiprofen (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4), and fexofenadine (P-gp). The probe drug doses used in this cocktail correspond to doses in the validated cocktail (Geneva cocktail), except for bupropion, where the lowest commercialized dose was selected.[17]

2. STUDY OBJECTIVES

Primary objective

- To evaluate a possible interaction (induction or inhibition) of St. John's wort dry extract Ze 117 on several cytochrome P450 enzymes and the transporter P-gp by comparing the primary pharmacokinetic parameters of the cocktail substrates .

Secondary objective

- To evaluate a possible interaction (induction or inhibition) of St. John's wort dry extract Ze 117 on several cytochrome P450 enzymes and the transporter P-gp by comparing the secondary pharmacokinetic parameters of the cocktail substrates and their relevant metabolites.
- To evaluate the safety of Ze 117 alone and in combination with the probe drugs.

Primary parameters: area under the plasma concentration-time curve up to the last sampling time with a concentration above the limit of quantification (AUC_{0-t}) at Days 1 and 17 for each of the probe drugs.

Secondary parameters: AUC_{0-t} and area under the plasma concentration-time curve extrapolated to infinity (AUC_{0-inf}), maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), terminal elimination rate constant (λ_z), elimination half-life ($t_{1/2}$) on Days 1, 8 and 17 for each of the probe drugs and their metabolites.

Safety variables: Adverse events, vital signs (blood pressure, pulse rate, body temperature), 12-lead ECG parameters, physical examination, and safety laboratory investigations.

3. ETHICS

3.1 ETHICAL CONDUCT OF THE STUDY

This study will be conducted in compliance with the Declaration of Helsinki[18] in the valid version and the International Conference on Harmonization (ICH) Guideline of GCP[19].

In addition, the European Union (EU) Clinical Trial Directives 2001/20/EC [20] and 2005/28/EC[21], the German Drug Law (Arzneimittelgesetz - AMG)[22], the Berufsordnung für Ärzte in Bayern, and the provisions of the data protection laws will be followed.

3.2 INVESTIGATOR OBLIGATIONS

The investigator agrees to conduct the clinical study in compliance with the protocol agreed with by the sponsor and, which was approved by the Ethics Committee and the Competent Authority (CA). The principal investigator and the sponsor should sign the protocol (and protocol amendments) to confirm this agreement.

3.3 INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW BOARD (IRB) AND COMPETENT AUTHORITY (CA)

Before the start of the study, CRO on behalf of the sponsor will apply for approval for the performance of the study at the CA (Federal Institute for Drugs and Medical Products

[BfArM]) and the Independent Ethics Committee (IEC) of the Bavarian Chamber of Physicians, which is constituted according to local law. All documents required in accordance with the German Drug Law by the IEC and by the CA will be submitted.

Any notification/submission has to be dated and to contain sufficient information to identify the respective protocol.

The study will only be started after receipt of the written approval of the IEC of the Bavarian Chamber of Physicians and the CA or, if the time of 30 days has elapsed, in case of implicit procedures.

The investigator and the sponsor are responsible for maintaining the approval documents in the study documentation files.

The investigator or the sponsor will report promptly to the IEC any new information that may adversely affect the safety of the subjects or the conduct of the trial.

If applicable, the sponsor (or legal representative of the sponsor) or CRO on behalf of the sponsor should submit a written report about the safety of the subjects as well as a list of occurred suspected serious adverse drug reactions (SAR) in the clinical study to the IEC and the CA annually, or more frequently, if requested by the IEC or the CA.

Declaration of the end of trial should be forwarded by CRO on behalf of the sponsor to the CA and to the IEC within 90 days after the study has been completed or in the event of premature termination of the study or interruption of the study within 15 days.

The sponsor (or legal representative of the sponsor) or CRO on behalf of the sponsor should provide a summary of the clinical study report to the IEC and CA within 1 year after completion of the study.

3.4 SUBJECT INFORMATION AND CONSENT

Prior to the start of screening examinations, the written informed consent form (ICF) must be signed and personally dated by the subject and by the investigator who conducted the informed consent discussion.

In obtaining and documenting informed consent, the investigator must comply with the applicable regulatory requirement(s), must adhere to GCP, and to the ethical principles that have their origin in the Declaration of Helsinki.

The investigator must fully inform the subject of all pertinent aspects of the trial including the written information approved/favorably assessed by the IEC. A subject should not be screened or treated until he has signed an approved ICF written in a language that is understandable to the subject.

The subjects should be informed of the possibility to withdraw consent without giving any reason. The information should include a statement that the consequence of the subject's withdrawal of consent will be that no new information will be collected from the subject and added to existing data or a database. The Investigator should maintain a log of all subjects who sign the ICF and indicate if the subject received study drug or, if not, the reason why. One original of the signed and dated ICF will be provided to the subject. A second original will be retained in the investigator site file.

The ICF will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change in the content of the subject information and/or the written ICF. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised ICF. This revised ICF must receive the IEC approval/favorable opinion in advance of use.

3.5 BENEFIT-RISK EVALUATION

Benefits:

There are no perceived direct benefits to healthy volunteers participating in this trial. However, thorough medical check-ups may be seen as an advantage.

The compliance with the eligibility criteria as well as regular assessments of the clinical status ensure that the safety is monitored closely and that both healthy volunteers and investigator have the opportunity to assess the continued participation in the trial, which can be discontinued at any time by the healthy volunteer.

A potential scientific benefit of this study would be to demonstrate that a low hyperforin content of Ze 117 may have less potential for drug interactions than other St. John's wort preparations and may therefore be more safely taken in co-administration with other essential medicines.

Risks:

On the other hand, the exposure to the medication may cause ill effects, discomfort and inconvenience.

Based on the safety profile of the product Remotiv® N 250 mg that is registered in Germany, the risks in participating in the trial are considered low. However, they include the usual risks of participating in clinical pharmacokinetic trials, which are related to blood drawing via venepuncture or indwelling catheter.

Following undesired side effects are known of the registered products Remotiv® N 250 mg (Germany) and Remotiv® extra 500 mg (Hungary):

- Nervous system disorders: dry mouth, headache, perspiration, asthenia, fatigue faintness, dizziness and agitation might appear

  Just like with other antidepressants following St. John's Wort extract treatment in some cases symptoms of hypomania or mania developed.

- Gastrointestinal disorders:

  stomach and intestinal disorders, gastrointestinal complaints

- Skin and subcutaneous tissue disorders: allergic reactions of the skin, phototoxic reaction of the skin.

  The St. John's Wort causes potentially light sensitivity.

  In literature, undesired reactions of the eyes were linked to the intake of St. John's wort preparations and concomitant sunlight exposure.

The drug cocktail consists of 7 probe doses. For the probe drugs marketed and commercially available drug in their dose levels will be used. No side effects after cocktail administration were reported by thirty volunteers participated in the cocktail validation study. None of the subjects reported side effects after single drug or cocktail administration.[17] There is extensive clinical experience of use of the probe drugs and full information about the potential side effects of the respective treatment.

General Safety

The study subjects will be carefully screened prior to study enrolment for their health status and suitability. Inclusion and exclusion criteria have been chosen appropriately to minimize possible risks for subjects participating in this trial. During the study the subjects will be closely monitored, including adverse event (AE) reporting, safety laboratory investigations, vital signs measurements and electrocardiogram (ECG). After study drug administration, the subjects will be under close medical surveillance. Subjects will be discharged on Day 18 from the study unit if there are no withstanding safety concerns. Any AE which remains unresolved requires detailed evaluation and follow-up until the AE has been resolved or a reasonable explanation for its persistence is found (see also Sections 9.1 and 9.8). In addition to this, subjects will receive an emergency card with information on their status and applied drug on leaving the study unit.

Blood drawing

The total amount of blood taken in this study is less than 300 mL, which will be taken over 20 days. In normal blood donation, up to 500 ml of blood is drawn within a single donation. Compared to a normal blood donation, there is no risk for volunteers.

Based on available information and the design of the trial, the sponsor and the investigator consider the trial to be ethically acceptable. The duration of hospitalization and the medical surveillance are considered adequate to ensure safety of the subjects.

In summary, the potential gain of knowledge put the potential risks of side effects of the investigational products in the healthy subjects during the study in relation:

Drug-drug interactions are a common safety problem during the treatment of antidepressive disorders.

Due to its low hyperforin content, Ze 117 may have less potential for drug interactions than other St. John’s wort preparations.

The aim of the study is to investigate the effect of Ze 117 on the interaction potential with relevant P450 (cytochrome P450) enzymes and P-pg transporter in order to provide a safe treatment of patients with depressive disorders afterwards.

This clinical study will be conducted in compliance with the clinical study protocol, Good Clinical Practice (ICH Topic E6, GCP) and the applicable regulatory requirements.

4. OVERALL STUDY DESIGN AND PLAN

4.1 OVERALL STUDY DESIGN

This is an open-label, non-randomized, single-sequence Phase I study.

Subjects will be hospitalized from Day -1 until discharge in the morning of Day 18. On arrival at the clinic, the review of restrictions will be performed and documented in the CRF. Subjects will be checked for items that are not allowed.

Subjects will receive 500 mg Ze 117 in the morning of Days 8 to Day 17. A cocktail consisting of 6 probe drugs will be given three times as a single dose each (Day 1, Day 8, Day 17):

The compilation of the probe drugs is selected according to the validated cocktail (Geneva cocktail) and contains the following probes:

- Caffeine (CYP1A2)
- Bupropion (CYP2B6)
- Flurbiprofen (CYP2C9)
- Omeprazole (CYP2C19)
- Dextromethorphan (CYP2D6)
- Midazolam (CYP3A4)
- Fexofenadine (P-gp)

Plasma samples for determination of the probe drugs and their metabolites will be taken until 72 hours after each cocktail administration. A final phone call will take place on Day 24 (±2 day) for follow-up.

Table 2 Study design

Day -21 to -2	Day -1	Day 1	Days 2 to 7	Day 8	Days 11 to 16	Day 17	Day 18	Days 19 and 20	Day 24 ± 2 days
Screening	Admis-sion	Dosing of probe drug cocktail on Day 1	Wash-out PK sampling until Day 4	Dosing of probe drug cocktail on Day 8	PK sampling until Day 11	Dosing of probe drug cocktail on Day 17	PK sampling until Day 20		Follow-up
				Ze 117 administration					
	Hospitalization period: Morning of Day -1 until Day 18 (24 hours after last dosing)							Ambu-lant visit	Follow-up phone call

4.2 OVERALL STUDY PLAN

For a detailed schedule of assessments, please see Table 1 of the study protocol.

4.2.1 Hospitalization

Subjects will be confined from Day -1 until Day 18.

4.2.2 Ambulatory Visits

Subjects will have an ambulatory visit at screening and on Days 19 and 20.

4.2.3 Screening Examination

Each subject must participate in the informed consent process and sign and date ICF before any procedures specified in this protocol are performed.

All subjects will undergo an entry examination to evaluate their health status. This examination will be conducted not more than 21 days prior to the planned first drug administration. Only subjects who meet the inclusion criteria and none of the exclusion criteria will be admitted to the study.

During the screening examination, the subjects are identified by a screening number.

Measures to prevent study participation within a time span defined by preceding study participation and to avoid participation in a study in parallel to another study:

There is a notification on the subject's card as well as in the electronic volunteer's data base on the last participation in a study. In addition, all subjects are reported to a central checking organization (VIP Check) before inclusion into the study.

This screening examination will consist of the following (for details of the measurements see Section 8.4.1):

- Medical history
- Physical examination
- Demographic information (age, sex, race, smoking status) including body height and body weight and body mass index (BMI)
- Safety laboratory: blood sampling (for clinical chemistry, hematology and coagulation) and urine sampling for safety laboratory (urinalysis sticks), including serum pregnancy test in female subjects
- Urine drug screen (including cotinine) and a alcohol breath test
- Serological tests for hepatitis B, C, and human immunodeficiency virus (HIV) 1/2
- Vital signs (supine blood pressure and pulse rate and body temperature)
- 12-lead ECG
- Adverse events
- Prior medication
- Evaluation of inclusion and exclusion criteria.

4.2.4 Study Performance

Day -1 (admission)

The subjects willing to participate in the study will only be included when all screening examination procedures have demonstrated that all inclusion criteria and none of the exclusion criteria apply. They will be assigned a randomization number within the study. For detailed information about the procedure of assigning randomization numbers to subjects please refer to Section 7.3.

Subjects will be hospitalized about 24 hours before the first drug administration (in the morning of Day -1). Thereafter, a single oral dose of the probe drug cocktail will be administered on Day 1, Day 8 and Day 17. Single doses of Ze 117 will be administered in the morning of Days 8 to 17. For details about study drug administration, please refer to Section 7. Subjects will be discharged from the Centre for Human Pharmacology of Nuvisan in the morning of Day 18.

All the following procedures will be performed.

The following activities will be performed prior to the first dose:

Day -1

- Vital signs (supine blood pressure, pulse rate and body temperature)
- 12-lead ECG
- Urine pregnancy test in female subjects
- Urine drug screen (including cotinine) and a alcohol breath test
- Re-check of in- and exclusion criteria
- Safety laboratory: blood sampling (for clinical chemistry, hematology and coagulation) and urine sampling for safety laboratory (urinalysis sticks),
- Prior medication
- Adverse events and concomitant medication

Days 1 to 4 (first probe drug administration)

The following activities will be performed before and after the first probe drug cocktail administration:

- Blood sampling for genotyping on Day 1 pre-dose
- Administration of the probe drug cocktail on Day 1
- Safety laboratory: blood sampling and urine sampling at 24 hours post-dose
- Vital signs (supine blood pressure and pulse rate, body temperature):
  on Day 1: pre-dose, 2, 4, 8 and 24 hours after dosing, on other days once in the morning, if applicable
- 12-lead ECG and physical examination at 24 hours post-dose
- PK samples for probe drugs and metabolites: pre-dose, and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after cocktail administration.
- Adverse events and concomitant medication

Days 5 to 7 (wash-out period)

- Adverse events and concomitant medication
- Vital signs (supine blood pressure and pulse rate, body temperature) in the morning, if applicable

Days 8 to 11 (second probe drug administration)

The following activities will be performed before and after the second probe drug cocktail administration:

- Administration of the probe drug cocktail on Day 8
- Administration of Ze 117 on Days 8 to 11
- Safety laboratory: blood and urine sampling on Day 8 pre-dose and 24 hours after administration of the probe drug cocktail
- Vital signs (supine blood pressure and pulse rate, body temperature):
  on Day 8: pre-dose, 2, 4, 8 and 24 hours after dosing, on other days once in the morning, if applicable
- 12-lead ECG and physical examination pre-dose and at 24 hours after administration of the probe drug cocktail
- PK samples for probe drugs and metabolites: pre-dose, and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after cocktail administration.
- Adverse events and concomitant medication

Days 12 to 16

- Administration of Ze 117 on Days 12 to 16
- Vital signs (supine blood pressure and pulse rate, body temperature):
  once in the morning, if applicable
- Adverse events and concomitant medication

Days 17 to 18 (third probe drug administration)

The following activities will be performed before and after the second probe drug cocktail administration:

- Administration of the probe drug cocktail on Day 17
- Administration of Ze 117 on Day 17
- Safety laboratory: blood and urine sampling pre-dose on Day 17 and 24 hours after administration of the probe drug cocktail
- Urine pregnancy test in female subjects 24 hours after administration of the probe drug cocktail
- Vital signs (supine blood pressure and pulse rate, body temperature):
  on Day 17: pre-dose, 2, 4, 8 and 24 hours after dosing
- Physical examination and 12-lead ECG on pre-dose on Day 17 and 24 hours after administration of the probe drug cocktail
- PK samples for probe drugs and metabolites: pre-dose, and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16 and 24 hours after cocktail administration
- Adverse events and concomitant medication

Subjects will be discharged after completion of the Day 18 activities.

Days 19 (ambulant visits)

- PK samples for probe drugs and metabolites 48 hours after cocktail administration
- Adverse events and concomitant medication

Days 20 - End of Study (ambulant visits)

The end of study examination has to verify that all values tested in the screening have remained within a clinically acceptable range. Unacceptable values and AEs will be followed up until they return to the reference ranges/resolved or there is an adequate explanation which is not related to the trial.

The end of study examination will consist of the following:

- Vital signs (supine blood pressure, pulse rate and body temperature)
- 12-lead ECG
- Physical examination
- Urine pregnancy test in female subjects
- Safety laboratory: blood sampling and urine sampling for safety laboratory
- PK samples for probe drugs and metabolites 72 hours after cocktail administration
- Adverse events and concomitant medication

Subjects will be asked for changes in their health state by trained staff of CRO to elicit information on adverse events (AE) on each study day (refer to Section 9.5). In case of AEs, the investigator might decide to perform/repeat a clinical examination and/or any laboratory tests (details are given in Section 9).

Dietary Regimen:

The subjects will receive standardized meals on Day -1 at customary times.

Dosing of the probe drug cocktail together with Ze 117 will be performed under fasting conditions, i.e. the subjects will be fasting for at least 10 hours (no food, no beverages, only water is allowed) before until 4 hours after administration of the probe drug cocktail on Days 1, 8 and 17. Four hours after drug administration, a standardized lunch will be served. An afternoon snack will be provided 7 hours and dinner 11 hours post-dose. Meals will be the same on Days 1, 8 and 17.

Dosing of Ze 117 on the other study days will be performed in the fasting state (fasting for at least 10 hours). Breakfast will be served about 1 hour after dosing of Ze 117 on these days.

On the other days meals will be served at customary times.

No water consumption will be allowed between 2 hour pre-dose and until 2 hour after dosing except for the fluid provided for the drug intake on Days 1, 8 and 17 (dosing of the probe drug cocktail together with Ze 117).

No water consumption will be allowed between 1 hour pre-dose and until 1 hour after dosing except for the fluid provided for the drug intake on Days 9 to 16 (dosing of Ze 117).

On other days no water intake restriction are required. Subjects will be advised to drink at least 1500 mL water on each hospitalization day.

Foods, sweets and beverages:

Intake of fruit juices, grapefruit/pomelo, starfruit, Seville oranges, tangelos Schisandra chinensis (Wu Wei Zi), golden seal (Hydrastis canadensis), cabbage, ginger, licorice root, broccoli, gingko, garlic will not be allowed for 7 days prior to admission until collection of the last PK blood sample.

Intake of methylxanthines (e.g. coffee, tea, cola, cacao, Rud bull) and alcohol containing food or beverages is not allowed from 24 hours prior to admission until collection of the last PK blood sample.

Poppy seeds (e.g. poppy seed rolls, poppy seed cake, yoghurt containing poppy seed etc.) will not be allowed 72 hours prior to admission until collection of the last PK blood sample.

Smoking:

Only non-smokers or former smokers, who have stopped smoking at least 3 months before study drug administration will be included in the study.

Exercise Regimen:

Subjects will be advised to avoid heavy physical exertion 48 hours prior to admission until collection of the last PK blood sample.

UV-exposure:

During the period of the study, intense UV-exposure should be avoided

4.2.5 End of Study

The follow-up call will be performed on Day 24 (± 2 days) to ensure the subject's wellbeing.

No medical treatment is planned after the end of the study. However, subjects exhibiting either subjective or objective abnormalities when the study has been completed will be followed up (see Section 8.4.1.4 for follow-up of laboratory values and Section 9.1 for follow-up of AEs). Any AE which remains unresolved after completion of the study requires detailed evaluation, follow-up, and if necessary specific medical treatment until the AE is resolved or a reasonable explanation for its persistence is found.

If the subject refuses to follow the instructions of the investigator, the latter is released from responsibility to follow-up.

The end of the study is defined as the last scheduled follow-up call of the last subject undergoing the study.

5. DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

This is a single center, non-randomized, open-label, fixed sequence study. The study design has been chosen in order to allow within-subject comparisons and thereby maximize the precision/power of the study and minimize the number of subjects required.

A fixed-sequence design has been selected as the most convenient for practical reasons. Oral administration is chosen for the study, as this is the clinical route of administration.

Administration of a cocktail is according to a validated method to simultaneously assess the activities of different CYP enzymes and the P-gp transporter.[17] CYP enzymes and transporter were selected on the basis on their PXR-mediated regulation.

Hyperforin (one of the constituents in St John's wort) has been demonstrated to be responsible for many of the observed clinical relevant drug interactions.

There is reasonable evidence to suggest that low-dose hyperforin extracts have no significant effects on cytochrome P450 (CYP) enzymes and transport proteins such as P-glycoprotein (P-gp). [12, 13]

Ze 117 will be administered for 10 days such that steady-state will be achieved and maintained to maximize any potential effect upon CYP and transporter. The probe drugs will be administered together with the first administration of Ze 117 and at steady state to assess CYP and P-gp activities in the presence of the inducer or inhibitors.

A single-center design is chosen for this trial to standardize the trial conditions and reduce variability.

This trial will be performed as an open-label trial. This is not considered to introduce bias, because the main assessment criteria are based on PK parameters.

Rationale regarding appropriateness of chosen gender distribution:

Healthy subjects will be recruited for the study as drug-drug interaction studies are usually conducted in healthy subjects unless PK is different between healthy subjects and patients.

Males and females will be included in equal parts into the study to evaluate sex-related differences in PKs. Possible differences between sexes will be evaluated in this study as the PK analysis is a within subject comparison as a subset analysis.

6. SELECTION OF STUDY POPULATION

The following inclusion and exclusion criteria are chosen to select the appropriate study population regarding homogeneity in order to meet the requirements for reliable evaluation of the data collected.

Inclusion and exclusion criteria will be checked at screening and will be rechecked, if applicable, on the day of participant's inclusion in the study (the day before drug administration).

6.1 INCLUSION CRITERIA

Participating subjects have to fulfill the following criteria:

1. Provide written informed consent to participate in the study and the genotyping after risks and benefits have been fully explained
2. Physically and mentally healthy as judged by means of a medical and standard laboratory examination
3. Caucasian male or female subjects aged between ≥18 and ≤55years at screening

4. BMI between ≥19 and ≤29 kg/m^2, and body weight ≤90 kg
5. Non-smoker (confirmed by negative urine continine test at screening and at admission)
6. If female, the pregnancy test at screening and at admission must be negative.
7. Subjects of childbearing potential must use an acceptable method of contraception / birth control and should be informed of the potential risks associated with becoming pregnant while enrolled within a clinical investigation. Acceptable methods for this study are: sexual abstinence or vasectomized sexual partner. Female subjects who are postmenopausal (no menses for at least 1 year) or surgically sterile (tubal ligation, hysterectomy or bilateral oophorectomy), diagnosed as infertile and not undergoing treatment to reverse infertility may be enrolled.

6.2 EXCLUSION CRITERIA

If any of the following applies the subject has to be excluded from the study:

1. Known or suspected hypersensitivity to any ingredient of the investigational medicinal product or any of the excipients (e.g. hereditary galactose intolerance, lactase deficiency or glucose-galactose malabsorption)
2. Subjects with a history of symptomatic orthostatic hypotension.
3. Any clinically significant illnesses or clinically significant surgery within 1 years prior to the administration of the study medication
4. Subjects with, or with a history of, any clinically significant neurological (especially myasthenia gravis seizure disorder), gastrointestinal (including ulcerative colitis, Crohn's disease, recurrent peptic ulcer or gastrointestinal hemorrhage), renal, hepatic, cardiovascular, psychiatric (including psychotic events like hallucinations, endogenous depression, suicidal ideation or suicidal behavior, bulimia or anorexia nervosa), respiratory (e.g. respiratory insufficiency, sleep apnea syndrome), metabolic, endocrine, hematological, dermatological or other major disorders (especially glaucoma) as determined by the Investigator.
5. Any surgical or medical condition which might interfere with absorption, distribution, metabolism or excretion of the drug
6. Any clinically significant abnormality or abnormal laboratory test results, as judged by the Investigator found during the medical screening, that in the opinion of the Investigator would preclude inclusion in the trial; estimated glomerular filtration rate (using the Modification of Diet in Renal Disease Study [MDRD] equation)[23] ≥ 90 mL/min/1.73^2 and/or liver function tests (alanine-amino-transferase [ALT] values above 1.1 x upper limit of normal (ULN), aspartate-amino-transferase [AST] values above 1.2 x ULN) confirmed by two repeated measurements, when it is checked during the screening laboratory tests[24]
7. Positive test of hepatitis B, hepatitis C or HIV screening (HIV antibody, Hepatitis B, Hepatitis C)

8. ECG abnormalities (long QT syndrome or QTcB > 450 msec (Bazett's formula), signs of ischemia, sinus tachycardia (HR > 90) or sinus bradycardia (HR < 50), ventricular conduct delay (QRS > 120 msec) or others, that are judged by the Investigator to be clinically significant at screening
9. Vital signs abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 60 or over 90 mmHg, or heart rate less than 50 or over 90 bpm) at screening
10. History of asthma or obstructive pulmonary disease (e.g. COPD)
11. Known photohypersensitivity
12. Subjects who consume more than 14 units of alcohol per week, or have a significant history of alcoholism as determined by the Investigator. Note: one unit of alcohol equals 285 mL of beer or lager, one glass [125 mL] of wine, or 25 mL of spirits.
13. Drinking excessive amounts of tea, cacao, chocolate, coffee, and/or beverages containing caffeine (> 500 mg/day). Note: 1 cup of coffee contains approximately 100 mg, 1 cup of tea approximately 30 mg, and 1 glass of coke approximately 20 mg of caffeine.
14. History of drug dependence or positive results of drug screen or alcohol breath test at screening or at admission.
15. Concomitant participation in another clinical trial within the 30 days prior to the planned first drug administration or previous enrolment into the current study
16. Donation of blood or blood products within 30 days prior to the planned first drug administration
17. Treatment with any medication, vitamins, natural food supplements (such as gingko or garlic preparations, Schisandra chinensis (Wu Wei Zi), golden seal (Hydrastis canadensis) or topical drug with systemic action used in the past 4 weeks prior to the planned first drug administration or 6 times the half-life of the respective drug, whatever is longer (excluding occasional use of paracetamol up to 24 hours prior to the planned first drug)
18. Suspected inability, e.g. language problems, or known or suspected non-compliances e.g. non-willingness to fulfill the study specific dietary restriction
19. Difficulty to swallow study medication
20. Enrolment of the Investigator, his/her family members, employees and other dependent persons
21. Any reason which, in the opinion of the Investigator, would prevent the subject from participating the study

6.3 REMOVAL OF SUBJECTS FROM THERAPY OR ASSESSMENTS

6.3.1 Removal of Subjects from Therapy or Assessment

A subject can decide to withdraw from the study participation at any time, for any reason, specified or unspecified, and without penalty or loss of benefits to which the subject is otherwise entitled. In this case, the subject must immediately contact the investigator and state that he is leaving the study.

The subjects should be informed of the possibility to withdraw consent without giving any reason and to require that all previously retained identifiable samples will be destroyed to prevent future analyses, according to national provisions. The information should include a statement that the consequence of the subject's withdrawal of consent will be that no new information will be collected from the subject and added to existing data or a database.

The investigator has to withdraw a subject from the trial in the following cases:

- occurrence of an AE which does not justify a continuation of the study
- impossibility to obtain samples
- protocol non-compliance judged as significant by the investigator, including non-compliance to the required trial considerations (e.g., food/diet requirements)
- protocol violation which jeopardizes the performance of the study

For further criteria see Section 6.3.4.

In the event of a subject deciding to stop participation in the study, he is requested to take part in the final medical examination including the required blood withdrawal (for the clinical laboratory tests). This final examination is for the subject's safety. This examination enables to detect any impairment to the subject's health, which may require treatment and could be related to the subject's participation in the study.

Replacement of drop-outs will be discussed between sponsor and Nuvisan. The data obtained from drop-outs will be used in the evaluation to the largest possible extent.

All safety data including medication concentrations until withdrawal will be collected, documented and reported. In case of subjects being withdrawn from the trial due to an AE or a serious adverse event (SAE), they will be asked to remain under supervision by the clinic until normal conditions are reached or otherwise qualified medical care is guaranteed.

Once withdrawn/dropped-out from the study, a subject may not re-enter the study.

6.3.2 Premature Discontinuation of the Study at a Trial Site

Not applicable to the present study.

6.3.3 Premature Discontinuation of the Complete Study

The sponsor may discontinue the complete study at any time, for ethical or scientific reasons, in agreement with CRO. The Principal Investigator is entitled at any time to stop

the study due to medical reasons. In such a case, the Principal Investigator should consult the sponsor at the earliest opportunity.

The study will be discontinued if one SAE occurred which is suspected to be drug-related i.e. is considered as one serious adverse drug reaction. If a relationship between an AE and the study drug(s) is at least reasonably possible, the AE is considered as drug-related.

6.3.4 Criteria for withdrawal/discontinuation of participants

Discontinuation comprises active withdrawals as requested by the subject or by the Investigator.

A subject may discontinue participation in the clinical trial at any time and for any reason, specified or unspecified, and without penalty or loss of benefits to which the subject is otherwise entitled.

Subjects may be withdrawn based on the Investigator's clinical judgement. Subjects must be withdrawn from the trial treatment by the Investigator if any of the following occurs:

- Occurrence of an AE of severe intensity (grade 3 or higher) which does not justify a continuation of the study or a SAE.
- Impossibility to obtain blood samples in general
- Protocol violation which jeopardizes the performance of the study
- Hypersensitivity reactions
- Moderate nausea lasting for more than 24 hours, more than 4 episodes of vomiting within 12 hours, or if the subject is prevented from eating: decreased oral intake for 24 – 48 hours, due to the before mentioned symptoms.
- Moderate abdominal pain, lasting more than 24 hours, or severe abdominal pain lasting more than 6 hours.
- Severe diarrhea (defined as unformed to watery stools of ≥ 6 stools per 24-hour period) for 2 consecutive days.
- No bowel movement within 5 days after last dosing
- Moderate or severe mental changes, including moderate sedation and somnolence lasting for more than 12 hours.
- Abnormal safety laboratory results, confirmed on ≥ 2 repeat measurements, including:
  - ALT or AST >5x ULN, alkaline phosphatase (ALP) >2.5x ULN or an increase in total bilirubin >2.5x ULN which are considered by the investigator to be possibly, probably or certainly related to the study drug.
  - Serum creatinine values >2x ULN which are considered by the investigator to be possibly, probably or certainly related to the study drug.
- Abnormal blood pressure confirmed on 2 or more measurements, including hypotension defined as systolic <80 mmHg and/or diastolic <40 mmHg, or hypertension defined as systolic >160 mmHg and/or diastolic >100 mmHg

(evaluated with the subject in supine position for a minimum of 5 minutes and confirmed by 2 repeat measurements. In case of repeat abnormal findings, cf. above, this should lead to direct study withdrawal.

- QTcB ≥500 ms and/or an increase of QTcB from baseline of ≥60 ms, confirmed by repeated ECGs, which is considered possibly, probably or certainly related to the study drug.
- Any clinically relevant symptom or sign which in the opinion of the investigator and/or sponsor warrant subject withdrawal.
- Request by the subject to discontinue (withdrawal of consent)

In the event of a subject deciding to stop participation in the study, he/she is requested to take part in the end of study examination including the required blood withdrawal (for the clinical laboratory tests). This end of study examination is for the subject's safety. This examination enables to detect any impairment to the subject's health, which may require treatment and could be related to the subject's participation in the study.

A subject who for any reason (e.g. failure to satisfy the selection criteria) terminates the study before administration of at least one dose of the study drug is regarded a 'screening failure'.

All safety data including medication concentrations until withdrawal will be collected, documented and reported. In case of subjects being withdrawn from the trial due to an AE or a serious adverse event (SAE), they will be asked to remain under supervision by the clinic until normal conditions are reached or otherwise qualified medical care is guaranteed.

7. TREATMENTS

7.1 TREATMENTS TO BE ADMINISTERED

Subjects will receive repeated oral doses of 500 mg Ze 117 for 10 days on Days 8 to 17.

The cocktail of 7 probe drugs will be given in the morning of Day1, Day 8, Day 17.

The selected probe drugs according to the validated Geneva cocktail are:

- Caffeine, 50 mg, tablet
- Bupropion HCl, 75 mg, tablet
- Flurbiprofen, 10 mg, oral solution
- Omeprazol, 10 mg, capsule
- Dextromethorphan, 10 mg, oral solution
- Midazolam, 1 mg, oral solution
- Fexofenadine, 25 mg, oral suspension

The probe drugs will be taken alone (Day 1) or together with Ze 117 (Days 8 and 17) together with 240 mL of water. The tablets will be taken first followed by the oral

solutions. Preparation and administration of the study drugs will be detailed in an IMP manual.

All drug administrations will be performed in accordance with the specifications of the investigator and under the supervision of Nuvisan staff members. Mouth check after each drug administration will be performed and respective documentation will be done in the CRF.

7.2 IDENTITY OF INVESTIGATIONAL PRODUCT(S)

Investigational product:

IMP1

Drug substance (medicinal product):	Ze 117 (Rebalance® 500; St. John's wort dry extract)
Formulation:	film-coated tablet
Administration:	oral
Strength:	500 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Zeller Medical AG, Romanshorn, Switzerland

Drug cocktail:

IMP2

Drug substance (Medicinal product):	Caffeine (Percoffedrinol® N 50 mg tablet)
Formulation:	tablet
Administration:	oral
Strength:	50 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Aristo Pharma GmbH, Berlin, Germany

IMP3

Drug substance (Medicinal product):	Bupropion (Bupropion hydrochloride)
Formulation:	film-coated tablet
Administration:	oral
Strength:	75 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Apotex Inc., Weston, Florida, USA

IMP4

Drug substance (Medicinal product):	Flurbiprofen (Froben 5mg/mL syrup)
Formulation:	solution (syrup)
Administration:	oral
Strength:	5 mg/mL
Dose	10 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	BGP Products S.r.l., Rome, Italy

IMP5

Drug substance (Medicinal product):	Omeprazole (Omeprazol AbZ 10)
Formulation:	gastro-resistant hard capsule
Administration:	oral
Strength:	10 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	AbZ-Pharma GmbH, Ulm, Germany

IMP6

Drug substance (Medicinal product):	Dextromethorphan (Bexin® Hustensirup)
Formulation:	solution (syrup)
Administration:	oral
Strength:	25 mg/10 mL (2.5 mg/mL)
Dose:	10 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Spirig HealthCare AG, Egerkingen, Switzerland

IMP7

Drug substance (Medicinal product):	Midazolam (Midazolam-ratiopharm® 2 mg/ml orale Lösung)
Formulation:	oral solution
Administration:	oral
Strength:	2 mg/mL
Dose:	1 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Ratiopharm GmbH, Ulm, Germany

IMP8

Drug substance (Medicinal product):	Fexofenadine (Children's Allegra® Allergy)
Formulation:	oral suspension
Administration:	oral
Strength:	27.97 mg/5 mL (5.594 mg/mL) Fexofenadine as 30 mg/5 mL (6 mg/mL) Fexofenadine HCl
Dose:	25 mg
Batch number:	to be noted in the trial master file
Expiration date:	to be noted in the trial master file
Marketing authorization holder :	Sanofi-Aventis U.S. (distributed by Chattem Inc., part of the Sanofi-Aventis group)

The sponsor will provide the CRO with Ze 117 manufactured and tested bulk ware according to applicable GMP requirements for clinical trial supplies together with a certificate of analysis in a packaging with specific labeling for EU import. The final study specific labelling according the GCP requirements will be performed by the CRO.

The CRO will purchase commercially available probe drugs. Preparation, storage, and use of the probe drugs will be done as detailed in the respective summary of product characteristics. [25], [26], [27], [28], [29], [30], [31]

Handling Requirements:

The investigator or pharmacist will be responsible for ensuring that the study drugs are stored in a locked area with limited access and in accordance with the instructions on the study medication labels.

The storage temperature in the refrigerator is monitored continuously by the Rees system.

Drug Accountability:

The CRO Clinical Trial Supplies (CTS) Department will document the receipt of the Ze 117 IMPs from the sponsor and delivery of these products to the Clinical Services Department of CRO. There, the responsible study coordinator will document the receipt of the study medications and record number of units of study drug used during the study. Used (e.g. empty bottles) and IMPs not used during the study performance will be sent back to the CTS Department.

The number of unused units of the IMPs will be documented by CRO CTS Department. According to the provisions of the sponsor, the IMPs will be destructed or sent back to sponsor.

7.3 METHOD OF ASSIGNING SUBJECTS TO TREATMENT GROUPS

Screening Number

Subjects will be assigned a screening number at clinical study entry (i.e., signing of informed consent). The screening numbers (number 501, and so on) will be sequential and rising.

Subject Number

Only subjects who comply with all selection criteria will be enrolled into the trial. Prior to the first administration the subjects will be given a subject number. The subject number will be documented on the CRF. The subject number will appear on all study documents relating to that subject.

The subjects will be assigned to the subject number according to the standard operational procedures (SOPs) of the CRO.

The investigator will keep a record relating the screening/subject numbers and the names of all subjects (including the CRO ID number) that have given their informed consent, to allow easy checking of data in subject files, when required. This record will also include the date of subject's enrolment, as well as subjects who could not be enrolled for whatever reason.

7.4 SELECTION OF DOSES IN THE STUDY

The dose of 500 mg Ze 117 selected for this study is the recommended daily dose of the active ingredient St. John's wort dry extract. The medicinal product is either taken twice a day (2 x 1 tablet of St. John's wort film coated tablets 250 mg) or once a day (1 x 1 tablet of St. John's wort prolonged-release tablet 500 mg) according to the recommended dosage in the HMPC monograph.[1]

The compilation of the probe drugs is composed according to the validated cocktail (Geneva cocktail).[17] For the probe drugs marketed and commercially available drug in their dose levels will be used.

7.5 SELECTION AND TIMING OF DOSE FOR EACH SUBJECT

Each subject will receive a single oral dose of the probe drugs on Days 1, 8 and 17. Repeated oral doses of Ze 117 will be administered in the morning of Days 8 to 17. The day of the first drug administration will be defined as Day 1.

The time of administration of the probe drugs will be considered as study time 0d0h (Day 1 0h, Day 8, 0h and Day 17, 0h) and will be reported in the case report form (CRF).

7.6 LABELLING AND PACKAGING OF THE INVESTIGATIONAL PRODUCT(S)

The investigational products of Ze 117 will be provided by the sponsor as blistered bulk medication. The CRO will purchase commercially available probe drugs.

The Clinical Trial Supply Department of Nuvisan will prepare and label the individual doses of Ze 117. Details will be given in an IMP manual.

The labeling requirements for the study medication which should be in accordance with Annex 13 and local requirements are: information on the sponsor (address, phone number), sponsor's study code, CRO (address, phone number), EudraCT-number, product name (active constituents, strength and dosage form), content by weight and number of units, route of administration, batch number, expiry date, subject number, dosing time (period relative study time), storage instructions and the term "Zur klinischen Prüfung bestimmt".

Each manufacturing/packaging process will be performed and documented in conformity with Good Manufacturing Practice. If necessary, internal drug names and batch numbers will be assigned.

7.7 BLINDING AND BREAKING THE BLIND

Not applicable, this is an open study.

7.8 PRIOR AND CONCOMITANT THERAPY

The use of other concomitant prescription or non-prescription medication (including nutritionals and life style drugs such as gingko or garlic preparations, Schisandra chinensis [Wu Wei Zi], golden seal [Hydrastis canadensis]) is strictly forbidden for the whole clinical period, i.e., from 4 weeks prior to the study drug administration until discharge except when necessary to treat an adverse event. In this case, e.g. single intake of paracetamol is allowed, however, only after consulting the investigator and obtaining approval.

In consultation with the sponsor, the investigator can decide to withdraw the subject from the trial when other concomitant medication is required or has been taken without consulting the investigator.

All concomitant medication taken during the study must be recorded in the CRF with indication, daily dose, route of administration, date and time of administration.

7.9 TREATMENT COMPLIANCE

During the treatment periods, drug administrations will be performed by a CRO staff member in accordance with the specifications of the investigator. The proper administration of the study medication will be documented on the individual CRF.

8. VARIABLES TO BE ASSESSED

8.1 EFFICACY VARIABLES

Not applicable to the present study.

8.2 PHARMACODYNAMIC VARIABLES

Not applicable to the present study.

8.3 PHARMACOKINETICS

8.3.1 Analytical Measurements

8.3.1.1 Time Points/Intervals for Sample Collection

Blood samples

Blood samples for analytical assay of the probe drugs and their metabolites will be collected by venous puncture or indwelling venous catheter as closely as possible to the following time points:

- pre-dose and 10, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after each cocktail administration (3 x 19 samples).

  It is acceptable if sampling will take place in a time window of ± 3min, for the time points starting 24 hours post dosing in a time window of ± 30min. Actual times of PK blood sampling will always be documented in the CRF as well as the deviations from the scheduled blood sampling time points.

The time of administration of the cocktail alone (Day 1) or together with Ze 117 (Days 8 and 17) will be defined as study time 0d0h. The date and the time of each sample collection will be recorded in the CRF.

The pre-dose sample will always be considered as if it had been taken simultaneously with the drug administration. Beyond this pre-dose convention, all pharmacokinetic calculations will be based on the actual times of the collection intervals as recorded.

A total of less than 300 mL of blood will be taken during this study.

8.3.1.2 Sample Handling Procedures

Information on blood sample handling, storage, and shipment will be provided to the Investigator in a laboratory manual.

All sample handling procedures, including the time of each sample collection, the time of placement into frozen storage (at the end of the sample workup), and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail.

All samples will be stored for a period of 6 months after submission of the final clinical study report to the sponsor. If no separate contract for further storage has been agreed upon by the sponsor and CRO, the samples will then be destroyed after approval by the sponsor.

8.3.1.3 Analytical Method(s)

Blood samples will be analyzed for the probe drug and metabolite concentrations using a validated LC-MS/MS method.

Analytes are:

- Caffeine and paraxanthine
- Bupropion and 4-hydroxybupropion
- Flurbiprofen and 4-hydroxyflurbiprofen
- Omeprazole and 5-hydroxyomeprazole

- Dextromethorphan and dextrorphan
- Midazolam and 1-hydroxymidazolam
- Fexofenadine

The assay will be carried out in accordance to Good Laboratory Practice regulations and the EMA reflection paper (GCP)[32]. A separate bioanalytical protocol will be provided before the start of the analytical part of the study. Characteristics of the analytical methods will be described in the clinical study report.

8.3.2 Pharmacokinetic Variables

8.3.2.1 Description of the Pharmacokinetic Parameters

The following non-compartmental PK parameters will be calculated from the individual blood concentration-time data of the probe drugs and their metabolites on Days 1, 8 and 17 using Phoenix WinNonlin 7.0 or later.

AUC_{0-t}	Area under the drug concentration-time curve, calculated using linear trapezoidal summation from time zero to time t_{last}, where t_{last} is the time of the last measurable concentration (C_t).
AUC_{0-inf}	Extrapolated area under the drug concentration-time curve from time zero to infinity based on the last predicted concentration, i.e., concentration at the final observation time estimated using the linear regression performed to estimate λ_z.
C_{max}	Maximum observed drug concentration.
λ_z	Apparent terminal elimination rate constant, calculated by linear regression of the terminal linear portion of the log concentration vs. time curve, where λ_z = - slope.
$t_{1/2}$	Apparent terminal elimination half-life, calculated as $\ln(2)/\lambda_z$.
t_{max}	Time of the maximum drug concentration (obtained without interpolation). If the maximum value occurs at more than one time point, t_{max} is defined as the first time point with this value.

8.3.2.2 Methods for Derivation of Pharmacokinetic Parameters

PK parameters will be calculated by so-called non-compartmental or model-free methods, e.g., linear trapezoidal rule for AUC, log-linear regression for λ_z etc.[33]

Actual sample times will be used in the calculations. Blood concentrations below the lower limit of quantification (LLOQ) will be treated as zero.

The PK parameters such as AUC_{0-t}, AUC_{0-inf}, C_{max}, t_{max} and $t_{1/2}$ will be computed, as the data permit.

Linear regression for determination of λ_z will be performed using at least 3 data points of the terminal elimination phase. λ_z will not be assigned if the terminal elimination phase is not apparent, or if the R^2 value is less than 0.8. In cases where the λ_z rate is not assigned, the values of AUC_{0-inf} and $t_{1/2}$ are not calculable and will not be reported.

The value of $AUC_{0\text{-inf}}$ will be considered as possibly unreliable but will be nevertheless reported if the terminal area beyond the last quantified sample is greater than 20% of the total $AUC_{0\text{-inf}}$.

8.3.2.3 Appropriateness of the Pharmacokinetic Parameters

The AUC is used to describe the extent of bioavailability. The rate of bioavailability is characterized by C_{max} and t_{max}. λ_z and t½ describe the kinetics in the terminal phase which, for many substances, is governed by elimination processes.

8.3.3 Pharmacogenomics

A blood sample for genotyping will be collected pre-dose on Day 1. Samples will be screened for polymorphisms of CYP in case of any abnormality in the pharmacokinetic profile.

Samples will be handled according to defined genotyping laboratory instructions included in the laboratory manual provided before the study starts.

Blood samples are stored until the end of the study (final Study Report). After that period, the Sponsor will be contacted to determine whether the samples will be forwarded to the Sponsor, destroyed or kept at another facility for longer period of time at the Sponsor's expenses.

8.4 SAFETY VARIABLES

8.4.1 Description of the Safety Variables

The safety variables to be assessed in the present study are AEs, vital signs, ECG recordings, and laboratory tests. Since vital sign measurements, laboratory tests, and ECG recordings will be performed for safety reasons only, deviations from the planned time schedule are not relevant; however, assessments should be performed as closely as possible to the planned time point.

8.4.1.1 Vital Signs

Blood pressure (systolic and diastolic) and pulse rate will be measured as scheduled in the study flow chart (Table 1). Measurements will be performed in the supine position after at least 5 minutes rest using an oscillometric method.

As far as possible, measurement will always be performed on the same arm.

Body temperature (ear) will be measured as scheduled in the study flow chart (Table 1).

8.4.1.2 ECG

12-lead ECGs will be recorded at time points given in Table 1. The ECGs will be recorded in supine position after at least 5 minutes rest.

The ECG should be interpreted by the investigator (normal/abnormal). For abnormal ECGs the clinical significance (yes/no) should be judged by the investigator and the abnormality is to be specified.

8.4.1.3 Physical Examination

Physical examinations will be performed as scheduled in the study flow chart (Table 1). These will include the examination of general appearance, skin, neck (including thyroid), throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological systems.

Any relevant findings are to be recorded on the Medical History form in the CRF (for findings from the past that occurred prior to first screening visit), or on the Adverse Events form in the CRF (for findings presently occurring; events existing but unresolved prior to drug administration).

8.4.1.4 Clinical Laboratory tests

Blood samples for hematology, biochemistry, coagulation and urine samples for urinalysis will be collected as scheduled in the study flow chart (Table 1).

Any abnormalities in any of the laboratory parameters will be judged by a physician individually in relation to the reference ranges from the laboratory.

All results will be judged by a physician individually and commented as follows:

- Values within the reference ranges will not be commented. A '*' representing the value will be plotted within the brackets representing the reference range.
- For values slightly outside the reference ranges without clinical relevance a '*' representing the value will be plotted outside the brackets representing the reference range.
- For values outside the reference ranges with major deviation and/or possible pathological relevance a '*' representing the value will be plotted outside the brackets representing the reference range. In addition, the respective parameter will be shaded.

For all findings with major deviation and/or possible pathological relevance, follow-up examinations will be carried out until the deviation returns to normal or the absence of pathological relevance can be confirmed. If a deviation considered possibly clinically relevant has not returned to a normal or not clinically relevant value when it is checked during the screening laboratory tests, the subject will not be included in the study.

Laboratory abnormalities considered clinically relevant by the investigator will be reported as AE.

The following parameters will be determined:

Hematology:

Hemoglobin, hematocrit, red blood count, mean cell hemoglobin, mean cell volume, mean cell hemoglobin concentration, platelet blood count, standard blood count and differential blood count (%, automated).

Clinical chemistry:

AST, ALT, GGT, ALP, total bilirubin, urea, creatinine, uric acid, creatine kinase, total protein, glucose, cholesterol, triglycerides, potassium, sodium, calcium, C-reactive protein.

Estimated glomerular filtration rate (using the Modification of Diet in Renal Disease Study [MDRD] equation):[23]

eGFR (mL/min/1.73 m^2) = 175 × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female)

Scr = serum creatinine

Coagulation:

Activated partial thromboplastin time, prothrombin time and INR.

Hormones:

Thyroid stimulation hormone (TSH, at screening only), free triiodothyronine and free thyroxine if TSH is out of range.

Urinalysis:

Nitrites*, leukocytes*, protein, glucose, ketones, urobilinogen, bilirubin, blood*, pH.

*In case of abnormal findings, an investigation of the urine sediment will be performed.

Laboratory exclusion tests:

Serology/virology: Human immunodeficiency virus antibodies 1/2, hepatitis B surface antigen and hepatitis C virus antibodies are performed only at screening for eligibility purposes.

Drug screen, alcohol breath test and cotinine test will be done at screening and on admission (Day -1).

Pregnancy test: Serum pregnancy test will be done at screening and urine pregnancy test on Day -1, on Day 18 and on Day 20 (end of trial examination)

The drug screening will be performed from fresh urine for the determination of: cotinine, amphetamines, methamphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, ecstasy, methadone, opiates, tricyclic antidepressants and phencyclidine.

8.4.1.5 Adverse events

AEs will be monitored continuously throughout the study, for details see Section 9.

8.4.2 Appropriateness of the Safety Variables

All safety measurements are performed according to standardized methods, which are widely used and generally recognized as reliable and accurate.

For eliciting and rating of AEs please refer to Sections 9.5 and 9.6, respectively.

9. ADVERSE EVENTS

9.1 ADVERSE EVENTS

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally

associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Untoward medical experiences occurring during drug-free screening period do not meet the above-mentioned definition of AE. Nevertheless, they have to be documented in the same way as AEs.

Treatment emergent AEs (TEAEs) are defined as any event not present prior to the initiation of the treatments or any event already present that worsens in either intensity or frequency following exposure to the treatments.

AE of special interest have not been defined.

During and following a subject's participation in this trial, the investigator has to ensure that adequate medical care is provided to a subject for any AEs, including clinically significant laboratory values, related to the trial.

Any AE which remains unresolved after completion of the trial requires detailed evaluation and follow-up until the AE has been resolved or a reasonable explanation for its persistence is found.

The subjects will be provided with cards stating that they are participating in a clinical study, giving the investigational product, the duration of the study, the address and telephone numbers of CRO. These cards are meant for the case that the subjects consult a physician during their study participation or for emergencies.

9.2 ADVERSE DRUG REACTIONS AND UNEXPECTED ADVERSE DRUG REACTIONS

An adverse drug reaction (ADR) is defined as any untoward and unintended responses to an investigational medicinal product related to any dose administered.

An unexpected ADR is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

9.3 SERIOUS ADVERSE EVENTS

A serious adverse event (SAE) is any untoward medical occurrence that, at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which 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 were more severe.

A hospitalization is not regarded as an SAE if the admission is pre-planned (i.e. elective or scheduled surgery arranged prior to the start of the study).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as 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. These should usually also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.4 SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR)

A serious adverse reaction (SAR) that is unexpected or for which the development is uncommon (unexpected issue) observed during a clinical trial and for which there is a relationship with the experimental drug. As this study uses a combination of IMPs, multiple SmPCs will be used to assess whether an SAR is unexpected (and therefore a SUSAR). The causality assessment will therefore be made on the combination rather than the individual IMPs. Therefore, if a ‘suspected’ SAR is not expected for any of the IMPs or occurs with increased severity for any of the IMPs, it will be required to be reported as a SUSAR.[34]

9.5 ELICITING, DOCUMENTATION AND REPORTING OF ADVERSE EVENTS

Information on AEs will be derived by questioning the subjects in general terms (e.g., "How do you feel?” or “How have you been feeling since the last questioning?"), by subjects' spontaneous reports, or by observation.

Adverse events will be documented on special source data sheets in German language. The entries on the source data sheets will be checked by trained CRO staff members. The AEs will be translated and transcribed to special CRF pages. The CRFs will be transmitted to the "Clinical Data Management" Department who will enter the AEs into a database for the further evaluation.

The following information will be given for each AE: Description of the AE, start date, start time, stop date, stop time, severity, action taken, outcome, seriousness, and possible relationship to the study drugs.

9.6 RATING OF ADVERSE EVENTS

Rating of AEs will be performed by the investigator.

The severity of AEs is characterized as follows:

mild: Any symptom, of which the subject is aware, but which is easily tolerated;

moderate: Any symptom, which is discomforting enough to cause interference with a subject's usual activity;

severe: Any symptom, which causes a subject's inability to perform usual activity.

The likelihood of drug causation is characterized as follows:

Relationship	Description
Certain	Event or laboratory test abnormality, with plausible time relationship to drug intake Cannot be explained by disease or other drugs Response to withdrawal plausible (pharmacologically, pathologically) Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon) Rechallenge satisfactory, if necessary
Probable / Likely	Event or laboratory test abnormality, with reasonable time relationship to drug intake Unlikely to be attributed to disease or other drugs Response to withdrawal clinically reasonable Rechallenge not required
Possible	Event or laboratory test abnormality, with reasonable time relationship to drug intake Could also be explained by disease or other drugs Information on drug withdrawal may be lacking or unclear
Unlikely	Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) Disease or other drugs provide plausible explanations
Not related	Causal relationship can be ruled out

The outcome of the adverse event should be classified according to the following definitions:

- Recovered
- Recovered with sequelae
- Recovering (the event or sequelae has stabilized)
- Not recovered
- Death

All efforts should be made to classify the AE according to the above categories.

Note: when the AE is ongoing, the outcome will remain blank on the Adverse Events form in the CRF.

9.7 REPORTING OF SERIOUS ADVERSE EVENTS (SAE) AND OTHER SAFETY RELATED EVENTS

Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor and the EU-QPPV of the study. The Sponsor will re-evaluate the SAE and return the form to the site.

SAEs resulting in death are reported to the local Ethics Committee (via local Investigator) within 7 days.

Reporting of SUSARs

A SUSAR needs to be reported to the local Ethics Committee (local event via local Investigator) and to the Competent Authority (in cooperation via with the Sponsor via QPPV) within 7 days, if the event is fatal, or within 15 days (all other events).

Reporting of safety signals

Reports will be addressed to the qualified person for pharmacovigilance

Name:	Dr. med. Werner Feuerer
Address:	Nuvisan GmbH, Wegenerstr. 13, 89231 Neu-Ulm, Germany
E-mail:	werner.feuerer@kabelbw.de
Mobile number:	+49 (0)170 2387 547 or +49 (0)170 2387 548
Phone number:	+49 (0)7351 577 3533
Fax:	+49 (0)7351 577 3534

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor within 24 hours. The Sponsor must report the safety signals within 7 days to the local Ethics Committee (local event via local Investigator) and to the Competent Authority via qualified person for pharmacovigilance.

Reporting and handling of pregnancies

Subjects who become pregnant during the study should discontinue the study immediately.

Nuvisan will notify any pregnancies to the sponsor within 24 hours after becoming aware of its occurrence.

Whenever possible a pregnancy should be followed to term, any premature terminations should be reported, and the status of the mother and child should be reported to the sponsor after delivery.

Periodic reporting of safety

An annual safety report is submitted once a year to the local Ethics Committee via local Investigator and to the respective Competent Authorities.

9.8 FOLLOW UP OF (SERIOUS) ADVERSE EVENTS

AEs and SAEs will be measured from screening until end of study visit. In case of ongoing AEs/SAEs at the end of study or if participants are terminating the study prematurely a follow up visit will be performed within the next 30 days to control whether AE/SAE has been resolved and to document the outcome properly into the CRF.

Any participant withdrawn prematurely from the study (for any reason) should have the assessments of the following visits performed:

- End of study visit as soon as possible after the last study medication administration

- Follow-up visit within 30 days (if AE is ongoing at end of study visit).

9.9 WARNINGS AND PRECAUTIONS

General precautions include those related to anaphylactic reactions of single individuals, which require emergency interventions by a physician including application of high dose corticosteroids, anti-histaminic drugs, volume resuscitation and catecholamine therapy as well as O_2-insufflation or general resuscitation in rare cases. As such, all testing facilities should have immediately available procedures to administer treatment for an anaphylactic response should it occur. In case of emergency however, the Investigator has to initiate any medical procedures, which he deems appropriate. All such procedures have to be reported immediately to the Sponsor.

During the trial, subjects will have the possibility to contact one of the responsible physicians of the clinical ward under a known telephone number.

10. QUALITY ASSURANCE AND QUALITY CONTROL

To ensure compliance with GCP and regulatory requirements, a member of the sponsor's (or a designated CRO's) quality assurance unit may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator/institution will be informed of the audit outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor immediately of any such inspection.

The investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study. All involved parties must keep the participant data strictly confidential.

10.1 MONITORING

The monitoring will be done by Nuvisan GmbH. On-site monitoring will be performed including Site Initiation, Interim Monitoring and Close out. The CRA will ensure that the trial is conducted, recorded, and reported in accordance with the protocol, SOPs, GCP, and the applicable regulatory requirements. The monitor will check the accuracy and completeness of the CRF entries against source documents, such subjects' medical history, demographic data, AEs and print-outs of ECGs and laboratory results. The investigator will provide direct access to source data/documents for trial-related monitoring.

A Monitoring Plan will be set up to specify study specific monitoring procedures in agreement with the sponsor.

10.2 AUDITS AND INSPECTIONS

Quality assurance and quality control systems are implemented and maintained using written standard operating procedures (SOPs) to ensure that the trial is conducted and data

are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

11. DOCUMENTATION, RECORD ACCESS AND RECORD KEEPING

11.1 DOCUMENTATION AT THE STUDY CENTRE

All data relating to the study will be documented in the CRF. This CRF is developed to record the data requested by the protocol. The investigator will ensure the accuracy, completeness, legibility, and timeliness of the data recorded in the CRFs. Any change or correction to a CRF will be dated, initialed, and explained (if necessary), and will not obscure the original entry.

Prior to the conduct of the study it will be defined which data will be documented on special source data sheets and then transferred to the individual CRFs and which data will be documented directly in the CRFs and regarded as source data.

The following data will be directly entered into the CRF and will be regarded as source data:

visit date, age, weight, BMI, ethnicity/race, vital signs, results of alcohol breath test, date and time of safety and PK blood sampling, inclusion/exclusion criteria, dosing details/drug label.

For all other data source are available at the investigator site (e.g. print-outs of results for laboratory values and ECGs).

A subject's participation in a study will be documented on his/her subject’s recruitment card. All studies performed at Nuvisan, which the subject has participated in to date are listed on this card.

At the beginning of the trial, Nuvisan documentation files will be established at the Centre for Human Pharmacology of Nuvisan. Nuvisan will maintain the trial documents as specified in the ICH Guideline of GCP and as required by the applicable regulatory requirements. Nuvisan will take measures to prevent accidental or premature destruction of these documents.

Nuvisan will permit trial-related monitoring, audits, and regulatory inspection providing direct access to source data/documents.

11.2 SUBJECTS’ DATA AND DATA PROTECTION

To protect the subject's identity, a subject identification code will be assigned by the investigator to each trial subject and used in lieu of the subject's name when the investigator reports AEs and/or other trial-related data. Personal information will be treated as confidential, but may need to be reviewed by authorized representatives of the sponsor (monitor and auditor, respectively) and regulatory authorities. The subject's consent to direct access to his/her original medical records for data verification purposes has to be obtained prior to a subject's participation in the trial.

The investigator must maintain a list of names and identifying information (e.g. screening number, randomization number, CRO subject identification code) of all subjects enrolled in the trial. The subject identification list will be kept by the investigator in the Investigator Site file.

11.3 DATA MANAGEMENT/CODING

The data management will be performed by the data management department of Nuvisan. The CRFs will be logged in, double data entry will be performed, and data will be checked using computerized and manual means to identify discrepancies. Queries will be issued, e.g., on missing data, inconsistencies, illegibility, abnormal values, and improperly corrected items (e.g., without initials, date of change or a clear reason for change). Answers to queries, which have to be signed by the investigator, will be implemented in the database.

AEs will be coded with the current version of Medical Dictionary for Regulatory Activities (MedDRA). Clinical laboratory data will be checked against the laboratory normal ranges. Quality control on the data will be performed on an ongoing basis during the study.

Clean data sets will be provided to the biostatistician to perform the statistical analysis.

After validation of the study report by the sponsor, the database will be transferred to the sponsor.

11.4 RECORD KEEPING/ARCHIVING

Essential documents of the clinical trial including the subject identification code list, source data and case report forms will be retained for 15 years after the completion or discontinuation of the trial at Nuvisan. After that period, the Sponsor will be contacted to determine whether the study records will be forwarded to the Sponsor, destroyed or kept at another facility for longer period of time at the Sponsor's expenses.

The CRFs will be sent to Max Zeller Söhne AG.

11.5 USE OF DATA AND PUBLICATION

In addition to the publication of clinical trial results by regulatory bodies, Max Zeller Söhne AG has the sole right to publish the results of the study, use the clinical trial results for registration purposes, internal presentation, including presentation to business partners, scientific meetings and – if the product is approved – for promotion.

If published the subjects' identity will not be revealed.

12. DATA EVALUATION

The statistical evaluation will be performed by Nuvisan. A Statistical Analysis Plan (SAP) will be written to provide details of the analysis. The SAP will be finalized before the database lock. Any deviations from the SAP will be justified in the clinical study report.

12.1 STATISTICAL HYPOTHESES

The primary parameter will be AUC_{0-t} at Days 1 and 17 for each of the probe drugs (i.e. the AUC_{0-t} at Days 1 and 17 for each of the probe drugs are co-primary).

In order to preserve the overall alpha level of 5% for this study a hierarchical testing strategy will be used.[35]

The null hypothesis will follow Schuirmann's two one-side tests procedure (α=0.05), i.e. assuming that there is an effect and the geometric mean ratio lies outside the limits.

The three different limits for the geometric mean ratio for AUC_{0-t} are:

(1) Mild effect limits (0.50 to 2.00) [35]

(2) Extended bioequivalence limits (0.7 to 1.43)

(3) Bioequivalence limits (0.8 to 1.25)

A 90% CI for the ratio of AUC_{0-t} which lies entirely within the limits provides evidence of at most mild effect/extended bioequivalence/bioequivalence of Ze 117 regarding inhibiting/inducing the respective probe drugs.

The testing will be performed for each probe drug for the mild effect limits followed by the extended bioequivalence limits and finally the bioequivalence limits.

The order will correspond to a hierarchical testing strategy[36] and therefore no adjustments for multiplicity will be performed.

The same hypotheses as for the primary parameter will be tested for the secondary pharmacokinetic parameters AUC_{0-t} (Day 1, 8 and 17), AUC_{0-inf} and C_{max} for probe drugs and metabolites.

Summary statistics will be provided for all pharmacokinetic parameters.

12.2 DETERMINATION OF SAMPLE SIZE

10 ± 1 male and 10 ± 1 female participants are planned to be included in this study.

The intra-subject coefficients of variation (CVs) for the AUCs of the administered cocktail probe drugs vary between 8% and 30% as reported in Bosilkovska et al 2016.[17]

A sample size of 16 subjects will allow a rejection of each null hypothesis "interaction present induction/inhibition" with $\alpha = 0.05$ (one-sided) and a power of at least 99% for a tolerance zone of 0.50 – 2.0 assuming as a conservative approach that the intra-individual CVs exceed 30% and the true ratio of $\mu test/\mu reference = 1.0$.

A sample size of 16 subjects will allow a rejection of each null hypothesis "interaction present induction/inhibition" with $\alpha = 0.05$ (one-sided) and a power of at least 90% for a tolerance zone of 0.70 – 1.43 assuming that the intra-individual CVs exceed 30% and the true ratio of $\mu test/\mu reference = 1.0$. Additional 4 subjects will be included to account for drop-outs as a safety margin, resulting in a total sample size of N=20.

Sample size calculations were performed using SAS 9.4 for equivalence of a one-sample mean in a multiplicative model.

12.3 STUDY SUBJECTS

12.3.1 Study Population

Safety set

Safety analysis will be performed on the Safety Set:

- all included subjects who have received one dose of study drug.

Pharmacokinetic set

The evaluation of PK data will be based on the PK Set, a subset of the Safety Set, which is characterized by the following criteria:

- the proper completion of the study at least up the end of PK sampling;
- the absence of any important protocol deviation which would impede the reliability of pharmacokinetic assessments.

Protocol deviations will be discussed between the sponsor and CRO and allocation of subjects to study populations will be fixed prior to database lock and prior to availability of bioanalytical data.

12.3.2 Protocol Deviations

Important deviations from the protocol, such as deviations from inclusion and exclusion criteria, deviations in sampling times will be reported in the clinical study report.

If an unexpected important deviation from the study protocol occurs, the investigator will consult the sponsor to make a decision how this deviation can be handled.

12.3.3 Demographic and Other Baseline Characteristics

Demographic variables and baseline physical characteristics (age, sex, race, weight, height, BMI, smoking status) will be summarized for the safety and the PK population, if different. The descriptive statistics will include number of observations, mean, standard deviation, median, minimum and maximum for continuous variables and number of observations and their percentages for categorical parameters.

In- and exclusion criteria ensure that participants are healthy and fit for the trial without any relevant concomitant or previous illness or treatment that might have an influence on study results. These data as well as medical history will also be listed.

12.3.4 Decoding of Randomization

Not applicable, this is an open study with a single sequence.

12.4 EFFICACY EVALUATION

Not applicable to the present study.

12.5 PHARMACODYNAMIC EVALUATION

Not applicable to the present study.

12.6 PHARMACOKINETIC EVALUATION

The PK analysis will be performed based upon the Pharmacokinetic Set. The primary focus of the statistical analysis is to describe and characterize the PK of the probe drugs and their metabolites.

The individual blood concentrations of the probe drugs and their metabolites will be listed and tabulated by treatment and time point for all subjects with at least one PK assessment.

Descriptive statistics for all PK parameters will be calculated by analyte. These tables will include number of observations, mean, standard deviation, median, minimum and maximum and additionally the geometric mean and geometric CV (not for t_{max}).

In order to assess the effect of multiple doses of Ze 117 on the primary parameter AUC_{0-t} of the probe drugs (and metabolites) on Day 17 one way ANOVA models with terms for subject and treatment on the log transformed (base e) parameters will be performed (probe drug alone [Day 1] or probe drug co-administered with Ze 117 [Day 17]). The estimated treatment differences between Day 17 and Day 1 will be transformed back to original scale and presented as a ratio between treatments including the corresponding 90% confidence interval.

A 90% confidence interval for the ratio of AUC_{0-t} which lies entirely within the limits of 0.80 to 1.25 provides evidence of no effect of inhibition/induction of Ze 117 on the respective probe drug.

A lower limit above 0.50 represents a mild induction and an upper limit below 2.00 a mild inhibition. (Note: As per the guideline an inhibitor increases plasma AUC and an inducer decreases the AUC).

The same model will be applied for the secondary parameters AUC_{0-t} on Day 8 and AUC_{0-inf} and C_{max} on Days 8 and 17 compared to Day 1.

12.6.1 Statistical/Analytical Issues

12.6.1.1 Summary of Evaluations

The PK parameters AUC_{0-inf}, AUC_{0-t} and C_{max} of the probe drugs and their metabolites will be assessed for drug interaction of Ze 117.

12.6.1.2 Adjustments for Covariates

No adjustments for covariates are necessary.

12.6.1.3 Handling of Dropouts or Missing Data

All data will be used to their maximum possible extent, but without any imputations for missing data. If a subject has appreciable or critical data points missing, he/she should be excluded from analysis. However, if a subject is missing an inconsequential data point, e.g. final collection time sample lost due to tube breakage and preceding sample unquantifiable low, then his/her remaining data can still be used.

12.6.1.4 Interim Analyses

Interim analyses are not anticipated.

12.6.2 Tabulation of Individual Data

Quality assured data will be provided by the bioanalytical lab. After release of the data by the responsible analyst, data are transferred for evaluation into SAS data sets.

The results of subject samples measured will be displayed in separate tables for each treatment, including descriptive statistics for concentrations at individual sampling times. Similar tables will be prepared for pharmacokinetic parameters.

12.6.3 By-subject Displays

For each subject concentration-time curves will be plotted on a linear and log-linear scale.

12.7 SAFETY EVALUATION

The safety evaluation will be based upon the Safety Set.

12.7.1 Statistical/Analytical Issues

The present study involves only relatively few subjects, and a formal comparative statistical analysis of safety data therefore is not reasonable. These data will be evaluated descriptively only. Details will be provided in the SAP.

12.7.1.1 Adjustments for Covariates

There will be no adjustments for covariates.

12.7.1.2 Handling of Dropouts or Missing Data

All safety data of subjects who received one of the IMPs will be included in the safety analysis. All data will be used to their largest possible extent without any attempt to impute or extrapolate missing data.

12.7.1.3 Interim Analyses and Data Monitoring

Accumulating safety data will be examined regularly for any changes that might constitute an AE or that might pose an unexpected hazard to the participants in the trial.

12.7.1.4 Multicenter Studies

Not applicable to the present study.

12.7.2 Extent of Exposure

All subjects will receive drugs at the pre-specified fixed dosages. Information relating to the extent of exposure is thus contained in the treatment labeling.

In case of drop-outs, the actual extent of exposure will be described verbally for each of the respective subjects.

12.7.3 Adverse Events

12.7.3.1 Display of Adverse Events

In the clinical database, adverse events will be encoded using the MedDRA dictionary, latest version, and any concomitant medication will be encoded with the World Health Organization (WHO)-Drug Dictionary, latest version.

When transcribing AE information from raw data, any AE that continues, but changed in intensity, will be considered as one and only one AE whereas a recurrent AE (e.g., a headache for a couple of hours each day) will be considered as several AEs.

12.7.3.2 Analysis of Adverse Events

Frequency tables will be compiled showing the number of subjects per treatment affected by one or more TEAEs and serious TEAEs, including percentages and the number of events.

Additionally, TEAEs will be tabulated by system organ class and preferred term. An TEAE will be referred to the treatment and time point after which it occurred, i.e. any AE with an onset before dosing will be counted as baseline AE and will be considered as TEAE only if the onset is after dosing (for definition of TEAE see Section 9.1).

12.7.3.3 Listing of Adverse Events by Subject

A listing of AEs according to the ICH Guidelines will be drawn up. This listing will contain, at minimum, a description of AEs as to nature, severity, time and date of occurrence, time lag since last dose of investigational drug, duration, treatment (if any), outcome and causality. Separate sorted listings, e.g., sorted by body system, are not considered necessary.

12.7.4 Deaths, Other Serious Adverse Events and Other Significant Adverse Events

Deaths, other SAEs, and other significant AEs will be reported in form of epicrisis. In case of deaths or other SAEs that evidently are not related to the subject's participation in the trial, e.g., traffic accident, the textual description of the AE may be shortened appropriately.

12.7.5 Clinical Laboratory Evaluation

Clinical laboratory examinations are performed during screening examinations in order to determine whether the subject is eligible for participation in the study. For all findings with major deviations and/or possible clinical relevance repeat examinations will be carried out until the deviation returns to the reference range or the absence of clinical relevance can be confirmed. Otherwise the subject will not be included in the study. Clinical laboratory examinations performed at the time of discharge are inspected for any relevant changes in health. If any such changes should occur, the investigator will decide whether they represent an AE which will be reported accordingly.

Laboratory results will be listed showing all data of a single examination of a specific subject. A separate listing will show all abnormal results considered possibly clinically relevant. Shift tables from baseline to Day 8 and Day 18 will be produced for the

categories normal, abnormal not clinically relevant and abnormal possibly clinically relevant.

12.7.6 Vital Signs, Physical Findings and Other Observations Related to Safety

Clinical data on blood pressure and pulse rate and body temperature as well as ECG results collected in the course of the study will be listed and summarized.

Other clinical data derived in the course of the study and entered into the database will be listed and summarized, as appropriate, any unusual finding will be commented upon in the report.

13. ADMINISTRATIVE PROCEDURES

13.1 AMENDMENTS TO THE PROTOCOL

Modifications to the valid protocol should be made as an amendment.

Substantial amendments should be signed by the principal investigator and the sponsor and approved by the CA and/or the IEC depending on the affected changes prior to being implemented, unless the amendment is made to eliminate an immediate hazard to the clinical study subjects.

Amendments are regarded as substantial where they are likely to have a significant impact on:

- the safety or physical or mental integrity of the subjects
- the scientific value of the trial
- the conduct or management of the trial
- the quality or safety of any IMP used in the trial

Non-substantial amendments (e.g., changes in telephone number, etc.) will not require approval by the CA and the IEC unless requested by the CA or IEC.

13.2 CONFIDENTIALITY AND PUBLICATION

The investigator agrees to keep strictly confidential all unpublished information and results concerning this trial. Unpublished information must not be published or disclosed without the sponsor's prior written approval.

13.3 REGISTRATION OF STUDY AND INVESTIGATORS

Before initiation of the study, Nuvisan will inform the Local Authority according to the German Drug Law about the trial.

A notification about the start and end of the study should be forwarded by the investigator to the Local Authority according to the § 67 German Drug Law.

13.4 INSURANCE, CONTRACTS AND FINANCES

The sponsor shall ensure that only inactive ingredients well known in pharmaceutical science and released by the respective national authorities for use in drugs and/or foodstuffs for humans will be employed in the manufacture of the investigational products. The sponsor shall further ensure that the investigational products are manufactured according to the WHO's revised GMP rules.

The Sponsor will arrange a trial-specific insurance prior to study commencement in accordance with applicable legislation, covering liability of the Sponsor and the Investigator(s) for damages that may occur in relation to the clinical trial. Particular reference will made in the subject information to the obligations of the insured subjects participating in the clinical trial, such as immediate notification of any injury that might have occurred during the clinical trial. For further relevant insurance conditions, please refer to the copy of the policy and conditions in the trial master file.

In addition to the protocol, trial-related duties, functions and financial aspects will be specified in the contract between the sponsor and Nuvisan as well as any other parties involved in the clinical trial.

14. REPORTING

After study completion, an "integrated" full report will be prepared according to the Note for Guidance on Structure and Content of Clinical Study Reports.[37]

The report should be compliant with e-CTD format.

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