Protocol

Title of trial: A placebo controlled, double-blind, randomised trial investigating safety, tolerability, pharmacodynamics, and pharmacokinetics after intravenous administration of FE 204205 in patients with cirrhotic portal hypertension
NCT number: NCT02929407
Sponsor trial code: 000249
Date: 17 January 2017

CONSOLIDATED CLINICAL TRIAL PROTOCOL INCLUDING NON-SUBSTANTIAL PROTOCOL AMENDMENT 01, 19 OCT 2016, AND SUBSTANTIAL PROTOCOL AMENDMENT 02, 17 JAN 2017

A placebo controlled, double-blind, randomised trial investigating safety, tolerability, pharmacodynamics, and pharmacokinetics after intravenous administration of FE 204205 in patients with cirrhotic portal hypertension

000249

EudraCT Number:	2016-001078-13
IND Number:	119,790
Investigational Medicinal Product:	FE 204205, solution for infusion
Indication:	Complications of cirrhotic portal hypertension
Phase:	1b
Name and Address of Sponsor:	Ferring Pharmaceuticals A/S Clinical Research & Development, Experimental Medicine Kay Fiskers Plads 11, 2300 Copenhagen S, Denmark Telephone number: +45 8833 8834
GCP Statement:	This trial will be performed in compliance with GCP.

SYNOPSIS

TITLE OF TRIAL

A placebo controlled, double-blind, randomised trial investigating safety, tolerability, pharmacodynamics, and pharmacokinetics after intravenous administration of FE 204205 in patients with cirrhotic portal hypertension

SIGNATORY INVESTIGATOR(S)

[redacted]

TRIAL SITE(S)

University of Barcelona, Hospital Clínic, Spain.

PLANNED TRIAL PERIOD	CLINICAL PHASE
First patient first visit: Q4 2016 Last patient last visit: Q1 2018	1b

BACKGROUND AND SCIENTIFIC JUSTIFICATION FOR CONDUCTING THE TRIAL

Complications of portal hypertension, including ascites, hepatorenal syndrome, and variceal bleeding, are leading causes of morbidity and mortality in cirrhosis. Cirrhosis is a common cause of death in adults worldwide. It results in approximately one million deaths per year worldwide, 170 000 per year in Europe, and 35 000 per year in the USA. Cirrhosis is the main indication for 5500 liver transplants each year in Europe.

Patients suffering from cirrhotic portal hypertension have an abnormally high splanchnic blood flow due to increased hepatic resistance and splanchnic arterial vasodilation. These circulatory disturbances cause a decrease in the effective arterial blood volume and a compensatory activation of the sympathetic nervous system, the renin-angiotensin-aldosterone system, and the non-osmotic release of vasopressin, which in turn results in fluid retention and renal vasoconstriction.

Terlipressin, a full V_{1A} receptor agonist, has been shown to be effective in the management of several complications to cirrhotic portal hypertension. Terlipressin reduces the portal pressure and this effect is mediated by an arteriolar vasoconstriction in the splanchnic area. By counter-acting the splanchnic arterial vasodilation and increasing the effective arterial blood volume, thereby improving renal perfusion and glomerular filtration rates, terlipressin has been shown to improve renal function in patients with hepatorenal syndrome. There are also reports showing that terlipressin, by normalising the underlying circulatory derangements, is effective in the treatment of refractory ascites. The rationale for use of terlipressin for treatment of variceal bleeding is to produce splanchnic vasoconstriction and thereby reduce portal blood inflow and portal pressure,

thus decreasing portal-variceal flow and pressure.

Existing V_{1A} receptor agonists, such as terlipressin, are full agonists and may produce excessive vasoconstriction if not dosed carefully. The risk profile of V_{1A} full agonists requires careful titration and monitoring to prevent the development of serious adverse events (SAEs) due to tissue hypoxia and ischemia. Thus, the major limitation of existing agents is a risk of excess vasoconstriction, restricting their use to short term applications in inpatient settings under close monitoring by specialists.

FE 204205 is a novel, selective vasopressin 1a receptor (V_{1A}) partial agonist in clinical development intended for the treatment of complications to cirrhotic portal hypertension. In comparison to full V_{1A} receptor agonists, the V_{1A} partial agonist FE 204205 is expected to have a substantially improved therapeutic index due to a lower maximal effect and lower risk for excessive vasoconstriction. In animal studies FE 204205 shows only approximately half of the maximal vasoconstriction produced by full agonists without any concomitant signs of ischemia (i.e. no increased lactate levels), which is assumed to result in adequate clinical vasoconstriction concomitant with the required safety.

Results from two Phase I studies, in which healthy subjects received FE 204205 intravenously, indicate that the maximal effect on vasoconstriction, by means of blood pressure, is reached at a plasma concentration of approximately 1 ng/ml, achieved by an intravenous infusion of 0.1 mg over 6 hours. The effect of the V_{1A} partial agonist FE 204205 appears to be capped compared to a full V_{1A} agonist, since increasing the dose 9-fold (from 0.1 mg to 0.9 mg over 6 hours) did not result in greater pharmacodynamic effects. Administration of 0.9 mg over 6 hours was well tolerated with an adverse events (AEs) profile related to the pharmacological effects of a V_{1A} receptor agonist.

Subcutaneous administration of FE 204205 resulted in the generation of an active metabolite (M1), characterised as a V_{1A} full agonist, at equimolar concentrations to FE 204205. The subcutaneous administration caused more AEs and of higher intensity than intravenous administration, and the maximal tolerated subcutaneous dose of FE 204205 was defined as 0.1 mg. After intravenous administration the M1 metabolite was detected only at very low concentrations, $\leq$2% of the FE 204205 concentration, and is not expected to show any appreciable pharmacodynamic effects in in this trial.

This is the first study with FE 204205 in patients with cirrhotic portal hypertension and the selected endpoints will focus on the evaluation of safety and tolerability of the treatment in general, the investigation of effects on portal and systemic haemodynamics, and the pharmacokinetics of FE 204205.

OBJECTIVES

- Safety and tolerability of single intravenous infusions of FE 204205
- Pharmacological effect of FE 204205 on systemic and portal haemodynamics in patients with cirrhotic portal hypertension
- Pharmacokinetics of FE 204205 after intravenous infusion
- Relationship between pharmacokinetics and pharmacodynamics of FE 204205
- Metabolites of FE 204205 in plasma and urine of patients
- Exploratory assessment of effects of FE 204205 on soluble biomarkers and renal function variables

ENDPOINTS

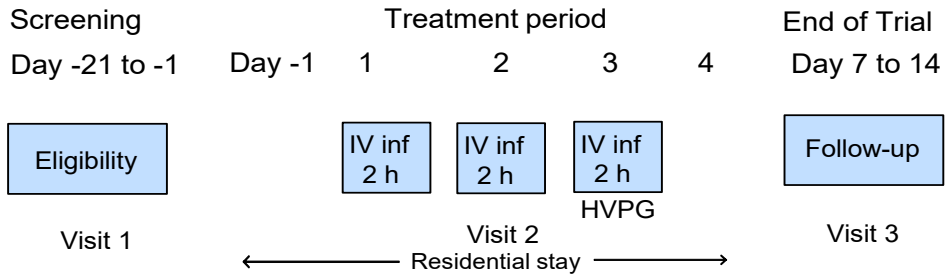
- Type, frequency and intensity of adverse events (AEs)
- Electrocardiogram (ECG) (intervals, rhythm, and morphology)
- Clinical chemistry, haematology and urinalysis
- Venous blood gases including lactate
- Systemic haemodynamics: Blood pressures and heart rate by non-invasive measurements, pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure (PAP), right atrial pressure (RAP), mean arterial pressure (MAP), and cardiac output (CO) by pulmonary artery catherization. CO, MAP, and RAP will be used for calculation of the systemic vascular resistance (SVR)
- Portal haemodynamics by hepatic vein catheterization: Free hepatic venous pressure (FHVP), wedged hepatic venous pressure (WHVP), and inferior vena cava pressure (IVC). WHVP and the FHVP will be used for calculation of the hepatic venous pressure gradient (HVPG)
- Pharmacokinetics FE 204205: AUC, AUC_t, %extrap AUC, C_{max}, t_{max}, CL, V_z, $t_{½}$, MRT, Ae, and CL_R
- Metabolites in plasma and urine
- Blood and urinary biomarkers: Renin, aldosterone, norepinephrine, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), urine creatinine, urine sodium, and urine osmolality

METHODOLOGY

This is a trial investigating the safety, tolerability, pharmacokinetics, and pharmacodynamics of FE 204205 in cirrhotic patients with portal hypertension and anticipated HVPG≥12 mmHg. The trial is divided in two parts:

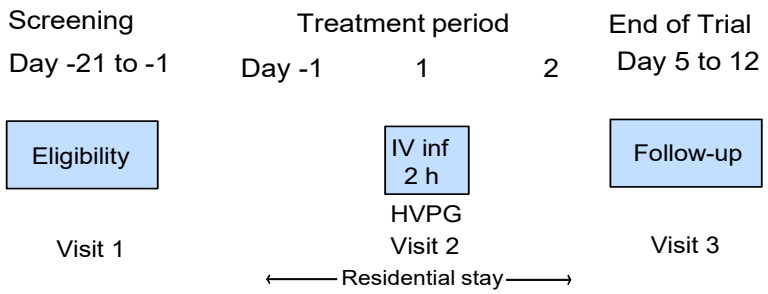
Part 1 is an open-label investigation of safety, tolerability, pharmacokinetics and pharmacodynamics of increasing intravenous doses of FE 204205 and will include 6 patients. The first two patients will receive their respective first dose on separate days. Systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization on Day 3. Each patient will receive three ascending doses of FE 204205, given once daily as 2-hour infusions on three consecutive days. Dose escalation on Days 2 and 3 will only commence provided that the increase in mean arterial pressure from baseline is <15 mmHg, absolute mean arterial pressure ≤120 mmHg, systolic blood pressure ≤160 mmHg, diastolic blood pressure ≤100 mmHg, and heart rate ≥55 beats per minute at any of the vital signs assessments during the 4 hours after start of infusion on the previous day. If these criteria are not met, the same dose will be administered the next day. If the increase of dose results in non-tolerable effects, the preceding tolerated dose will be reverted to for the remaining administrations in that patient.

For the first 3 patients, the initial treatment given on Day 1 will be a single dose of 0.01 mg FE 204205 followed by planned doses of 0.025 mg FE 204205 on Day 2 and 0.05 mg FE 204205 on Day 3. For the remaining 3 patients, the planned single doses are 0.025 mg FE 204205 on Day 1, 0.05 mg FE 204205 on Day 2 and 0.1 mg FE 204205 on Day 3. After the first 3 patients have completed all Visit 2 assessments, a Safety Review Committee (SRC) with at least one external expert will evaluate the safety, tolerability, and pharmacodynamics of the compound and will decide if the dosing regimen can be increased as planned, or if a modified (lower) dose regimen should be applied.



A Safety Review Committee (SRC) with at least one external expert, will evaluate the safety, tolerability, and pharmacodynamics of the compound after the completion of Part 1 to recommend what dose of FE 204205 to evaluate in part 2.

Part 2 is a placebo controlled, double-blind, randomised investigation evaluating the effects of a single dose of FE 204205 on portal haemodynamics and will include 16 patients receiving FE 204205 and 4 patients receiving placebo. Each patient will receive a 2-hour intravenous infusion of the maximum tolerated dose of FE 204205 as defined in Part 1, or placebo, on Day 1. Systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization.



NUMBER OF SUBJECTS

Part 1

6 patients. Subjects withdrawn prematurely in Part 1 will be replaced in order to achieve 6 subjects that complete all Visit 2-assessments.

Part 2

Approx. 20 patients in Part 2. At least 18 patients in Part 2 with HVPG ≥12 mmHg. Measures will be taken to reach a balanced ratio between genders (i.e. at least 1/4 of each gender) in the study.

CRITERIA FOR INCLUSION / EXCLUSION

Patients, 18-65 years of age, with cirrhotic portal hypertension and anticiptated HVPG ≥12 mmHg

Inclusion criteria

1. Signed written informed consent
2. Male or female patient, 18-65 years of age with confirmed evidence of cirrhosis
3. From medical history anticipated HVPG ≥12 mmHg
4. Negative pregnancy test at Screening and on Day -1
5. Agrees to use an adequate method of contraception during the study until the follow-up visit, if not abstinent. Adequate methods of contraception include condom with or without spermicidal gel (adequate only for male participants), intrauterine device, surgical sterilisation, vasectomy, oral contraceptive pill associated with inhibition of ovulation, depot progesterone injections
6. Negative urine drug screen and alcohol urine test at Screening and on Day -1

Exclusion criteria

1. Patients with co-existing disease including but not restricted to significant organ failure, decompensated cirrhosis with a Child Pugh score >12 or requirement for organ support, and acute pancreatitis
2. Type 1 hepatorenal syndrome
3. Severe hyponatremia (serum sodium concentration <125 mEq/L)
4. Acute-on-chronic liver failure
5. Hepatic encephalopathy ≥grade 2

6. Hepatocellular carcinoma outside the Milan criteria (<5 cm for a single lesion or less than 3 lesions with the largest measuring ≤3 cm)
7. Bacterial infection within 7 days prior to dosing
8. Gastrointestinal bleeding within 6 weeks prior to dosing.
9. Severe renal impairment i.e. estimated creatinine clearance < 30mL/min calculated according to the The Modification of Diet in Renal Disease (MDRD) study equation
10. History of underlying chronic heart disease, including but not restricted to heart failure NYHA class III or IV, angina pectoris, or myocardial infarction
11. Diagnosed ongoing hypertension
12. Symptomatic peripheral vascular disease including Raynaud's syndrome
13. Diagnosed chronic obstructive pulmonary disease (COPD)
14. Severe grade 3 anemia, i.e. Hb < 8.0 g/dL (< 4.9 mmol/L)
15. Use of vasopressin or terlipressin within 7 days prior to dosing
16. Permanent use of beta-blocker or a long-acting so called nitro-dilator, or if temporary use within 7 days prior to dosing
17. Use of treatments for hepatitis B or C virus within 6 months of randomisation or anticipated use during the trial period
18. Positive serology for human immunodeficiency virus (HIV)-1 or HIV-2
19. Women being pregnant or breastfeeding at screening and Day -1
20. Hypersensitivity towards any component of FE 204205 formulation
21. Sensitivity to contrast media
22. Intake of an investigational medicinal product within the last 8 weeks preceding screening or longer if judged by the investigator to possibly influence the outcome of the current study
23. Body mass index (BMI) <18 or >35 kg/m^2
24. Abuse of alcohol or drugs within 6 months prior to screening
25. Mental incapacity or language barrier precluding adequate understanding or co-operation
26. Previously included in this trial
27. Considered by the investigator to be unsuitable to participate in the study for any other reason

MEDICINAL PRODUCTS

Investigational medicinal products (IMP)

FE 204205: 10 mg/mL in 10 mM acetate buffer, pH 4.5, with mannitol to isotonicity

Placebo: Sodium chloride 0.9%

Non-investigational medicinal products (non-IMP)

Sodium chloride 0.9% for dilution of IMP
Midazolam
Mepivacaine
Iodine

DURATION OF TREATMENT

In part 1, each patient will receive three ascending doses of FE 204205, given once daily as 2-hour infusions on three consecutive days. In part 2, each patient will receive a single 2-hour intravenous infusion of FE 204205 or placebo.

TRIAL PROCEDURES / ASSESSMENTS

Part 1:
Before inclusion into the study, all patients will undergo a general physical examination, including vital signs, ECG and laboratory assessments including haematology, clinical chemistry, and urinalysis.

Patients will come for a residential stay, lasting between Day -1 to Day 4. Each patient will receive three ascending doses of FE 204205, given as 2-hour infusions over three consecutive days. During the treatment period, adverse events, vital signs, ECG, body weight, and clinical laboratory parameters will be assessed. Blood samples for measurement of biomarkers and plasma concentrations of FE 204205 and metabolites will be collected at specified time-points. Urine collection will be performed at specified timepoints for analysis of urinary output, urinary biomarkers and excretion of FE 204205 and metabolites. Patients will be confined to the clinic during the treatment period and will be carefully monitored for adverse events and effects on systemic haemodynamics. Systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization with measurements performed before, during, and immediately after the intravenous infusion on Day 3. Catheterization of the hepatic vein and the pulmonary artery is a moderately invasive technique and will be carried out under mild sedation with midazolam and local anaesthesia.

HVPG will be the primary pharmacodynamic parameter and assessments will be performed pre-dose, and at 1 and 2 hours after start of the intravenous administration. HVPG is the golden standard for evaluation of portal hypertension in patients with cirrhosis. A balloon-tipped catheter is placed in the right or middle hepatic vein and the FHVP and WHVP are measured, the latter is when the vein is occluded by the balloon. All measurements will be performed at least in duplicate (the average will be used for the endpoint), and permanent tracings will be obtained with a multichannel recorder and adequately calibrated transducers. The HVPG is calculated as the difference between the WHVP and the FHVP. Hepatic vein catheterization is a safe procedure and is used routinely in clinical practice in patients with chronic liver disease. In association with the hepatic vein catheterization, inferior vena cava pressure (IVC) will be measured pre-dose and at 2 hours after start of the intravenous administration.

Immediately before and after the hepatic vein catheterization (i.e. pre-dose and at 2 hours after start of the intravenous administration) a Swan-Ganz catheter will be introduced into the pulmonary artery for measurements of systemic haemodynamics (pulmonary capillary wedge pressure [PCWP], pulmonary artery pressure [PAP], right atrial pressure [RAP], mean arterial pressure [MAP], and cardiac output [CO]). CO, MAP, and RAP will be used for calculation of the systemic vascular resistance [SVR].

A follow-up visit will be performed between Day 7 and Day 14.

Part 2:
Before inclusion into the study, all patients will undergo a general physical examination, including vital signs, ECG and laboratory assessments including haematology, clinical chemistry, and urinalysis.

Patients will come for a residential stay, lasting between Day -1 to Day 2. Each patient will receive a 2-hour intravenous infusion of FE 204205 or placebo on Day 1. During the treatment period, adverse events, vital signs, ECG, body weight, and clinical laboratory parameters will be assessed. Blood samples for measurement of biomarkers and plasma concentrations of FE 204205 and metabolites will be collected at specified time-points. Urine collection will be performed at specified timepoints for analysis of urinary output, urinary biomarkers and excretion of FE 204205 and metabolites.

On Day 1, systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization.

A follow-up visit will be performed between Day 5 and Day 12.

STATISTICAL METHODS

Power

Part 1: No formal sample size calculation has been made. However, the number of patients (6) is considered sufficient to provide adequate information about the safety and tolerability of FE 204205, and thus inform about the suitable dose to evaluate in part 2.

Part 2: Since this is a first exploratory evaluation of the efficacy of FE 204205 on reducing portal pressure, the sample size and power calculations were performed by evaluation of the intra-group difference (before and after treatment with FE 204205). Assuming, based on the literature, a standard deviation (SD) of relative change from baseline of 12 %, a sample size of 14 patients on active treatment was deemed sufficient to detect a within-group 10 % reduction in HVPG, with $\alpha = 0.05$ and $1-\beta = 0.8$. Taking into consideration that it occasionally can be technically challenging to assess HVPG, a total of approx. 20 patients (16 on FE 204205 and 4 on placebo) are targeted (4:1 randomisation).

Statistical analyses

The statistical analysis will include descriptive statistics reflecting the explorative nature of the study. In general, the data will be presented by dose level in Part 1 and by treatment group in Part 2.

No single primary endpoint has been identified, and there will be no multiplicity adjustments for the statistical analysis comparisons performed. In Part 2, the statistical analyses are based on the assumption that a placebo effect is negliable, so the primary statistical analyses will be performed using analyses of covariance of the change and relative change from baseline in the pharmacodynamic parameters adjusting for the respective baseline values to assess the significance of comparisons with baseline within each group (in the treatment group to assess the treatment effect and in the placebo group to assess the assumption of a negliable placebo effect). Secondary inferential statistics will be computed between placebo and active treatment using an analyses of covariance with treatment group as an additional factor.

The pharmacodynamic and safety endpoints will be analysed for the safety analysis set. The PK analyses will be performed for the PP analysis set.

HVPG will be derived as the average of the duplicte assessments, separately calculated for each scheduled assessment, of the difference between WHVP and FHVP (WHVP- FHVP).

HVPG and other haemodynamic variables will be assessed as change from baseline to 1 and 2 hours post dose separately analysed for each treatment group, and compared between groups.

Non-compartmental pharmacokinetic analysis of FE 204205 will be performed.

Changes in safety parameters will be evaluated by subject listings and descriptive statistics.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

List of Abbreviations

AE	Adverse event
ATC	Anatomical therapeutic chemical classification system
CO	Cardiac output
ECG	Electrocardiogram
eCRF	Electronic case report form
EudraCT	European Union Clinical Trial Database
FHVP	Free hepatic venous pressure
FPFV	First patient first visit
GCP	Good clinical practice
GMP	Good manufacturing practice
HVPG	Hepatic venous pressure gradient
ICH	International Conference on Harmonisation
IEC	Independent ethics committee
IMP	Investigational medicinal product
ITT	Intention-to-treat
IVC	Inferior vena cava
LLOQ	Lower limit of quantification
LPLV	Last patient last visit
MAP	Mean arterial pressure
MedDRA	Medical Dictionary for Regulatory Activities
Non-IMP	Non-investigational medicinal product
PAP	Pulmonary artery pressure
PCWP	Pulmonary capillary wedge pressure
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Per protocol
RAP	Right atrial pressure
SAE	Serious adverse event
SRC	Safety review committee
SVR	Systemic vascular resistance
WHVP	Wedged hepatic venous pressure

Definition of Terms

Definition of general terms

Enrolled	When subject and Investigator have signed the Informed Consent form
Included	When the subject has received the first dose of IMP
Pre-dose	Before dosing on a day of dosing
Treatment period	Part 1: Time from start of infusion on Day 1, until 24 hours after the last administration. Part 2: Time from administration until 24 hours after administration

Pharmacokinetic terms and definitions

AUC	Area under the concentration-time curve to infinity
AUC_t	Area under the concentration-time curve from time zero up to time t, where t is the last time point at which the concentration is above the lower limit of quantification
% Extrap AUC	Percentage of AUC that is due to extrapolation from the last measurable concentration
C_{max}	Maximum concentration observed
t_{max}	Time of maximum observed concentration (C_{max})
CL	Total systemic clearance
CL_R	Renal clearance
V_z	Volume of distribution associated with the terminal phase
λ_z	First-order rate constant associated with the terminal (log-linear) portion of the concentration-time curve
t½	Elimination half-life
MRT	Mean residence time
Ae	Cumulative amount of FE 204205 excreted unchanged in the urine

1 INTRODUCTION

1.1 Background

Complications of portal hypertension, including ascites, hepatorenal syndrome, and variceal bleeding, are leading causes of morbidity and mortality in cirrhosis. Cirrhosis is a common cause of death in adults worldwide. It results in approximately one million deaths per year worldwide, 170 000 per year in Europe, and 35 000 per year in the USA. Cirrhosis is the main indication for 5500 liver transplants each year in Europe (1).

Patients suffering from cirrhotic portal hypertension have an abnormally high splanchnic blood flow due to increased hepatic resistance and splanchnic arterial vasodilation. These circulatory disturbances cause a decrease in the effective arterial blood volume and a compensatory activation of the sympathetic nervous system, the renin-angiotensin-aldosterone system, and the non-osmotic release of vasopressin, which in turn results in fluid retention and renal vasoconstriction (2).

Terlipressin, a full V_{1A} receptor agonist, has been shown to be effective in the management of several complications to cirrhotic portal hypertension (3). Terlipressin reduces the portal pressure and this effect is mediated by an arteriolar vasoconstriction in the splanchnic area. By counter-acting the splanchnic arterial vasodilation and increasing the effective arterial blood volume, thereby improving renal perfusion and glomerular filtration rates, terlipressin has been shown to improve renal function in patients with hepatorenal syndrome (4). There are also reports showing that terlipressin, by normalising the underlying circulatory derangements, is effective in the treatment of refractory ascites (5). The rationale for use of terlipressin for treatment of variceal bleeding is to produce splanchnic vasoconstriction and thereby reduce portal blood inflow and portal pressure, thus decreasing portal-variceal flow and pressure (3).

Existing V_{1A} receptor agonists, such as terlipressin, are full agonists and may produce excessive vasoconstriction if not dosed carefully. The risk profile of V_{1A} full agonists requires careful titration and monitoring to prevent the development of serious adverse events (SAEs) due to tissue hypoxia and ischemia. Thus, the major limitation of existing agents is a risk of excess vasoconstriction, restricting their use to short term applications in inpatient settings under close monitoring by specialists.

FE 204205 is a novel, selective vasopressin 1A receptor (V_{1A}) partial agonist in clinical development intended for the treatment of complications to cirrhotic portal hypertension. FE 204205 have a peptide sequence that comprises one V_{1A} agonist part linked to a V1A antagonist part, resulting in a molecule with partial V_{1A} agonist properties, thereby improving the therapeutic index by a lower maximal effect and lower risk for excessive vasoconstriction. In animal studies FE 204205 shows only approximately half of the maximal vasoconstriction produced by full agonists without any concomitant signs of ischemia (i.e. no increased lactate levels).

Results from two Phase I studies, in which healthy subjects received FE 204205 intravenously, indicated that it acts as a partial V_{1A} agonist, resulting in adequate clinical vasoconstriction

concomitant with the required safety. The the maximal effect on vasoconstriction, by means of blood pressure, was reached at a plasma concentration of approximately 1 ng/ml, achieved by an intravenous infusion of 0.1 mg over 6 hours. The effect of FE 204205 appears to be capped compared to a full V_{1A} agonist, since increasing the dose 9-fold (from 0.1 mg to 0.9 mg over 6 hours) did not result in greater pharmacodynamic effects. Administration of 0.9 mg over 6 hours was well tolerated with adverse events (AEs) as expected by a V_{1A} receptor agonist.

Subcutaneous administration of FE 204205 resulted, possibly due to local peptidase activity, in the generation of an active metabolite (M1) at equimolar concentrations to FE 204205. M1 is characterised as a V_{1A} full agonist, and the subcutaneous administration caused more AEs and of higher intensity than intravenous administration, and the maximal tolerated subcutaneous dose of FE 204205 was defined as 0.1 mg. In contrast, after intravenous administration the M1 metabolite was detected only at very low concentrations, ≤2% of the FE 204205, and is not expected to show any appreciable pharmacodynamic effects in in this trial.

This is the first study with FE 204205 in patients with cirrhotic portal hypertension and the selected endpoints will focus on the evaluation of safety and tolerability of the treatment in general, the investigation of effects on portal and systemic haemodynamics, and the pharmacokinetics of FE 204205.

1.2 Scientific Justification for Conducting the Trial

This is the first study with FE 204205 in patients with cirrhotic portal hypertension, and the selected endpoints will focus on the evaluation of safety and tolerability of the treatment in general, the effects on portal and systemic haemodynamics, and the pharmacokinetics of FE 204205.

1.3 Benefit / Risk Aspects

FE 204205 is a partial V_{1A} vasoconstrictor and the pharmacological effects observed in healthy subjects include increased blood pressure. A possible benefit of participating in this study would be that FE 204205 shows good effect in decreasing the portal pressure, thus rendering the patient a positive, albeit possibly temporary, clinical outcome.

However, the mechanism of action may also pose some risks. Effects observed in healthy subjects include peripheral vasoconstriction, decreased organ perfusion, bradycardia, and decreased cardiac output. The observed AEs included abdominal pain, increased bowel activity, nausea, increased blood pressue, and decreased heart rate.

In the one patient with cirrhosis dosed with FE 204205 to date (patient [redacted] in trial 000249), severe arterial hypertension and severe diarrhoea were reported. After review of the respective patient´s data, the Safety Review Committee concluded that it would be safe to continue the trial with a modified and lower dosing regimen as well as an increased frequency of blood pressure and heart rate monitoring (changes incorporated with Amendment 2).

Based on the pharmacological effects of FE 204205, cardiovascular monitoring of the trial subjects will receive special attention. The trial is conducted in a hospital ward for patients with liver disease with close medical supervision. The Principal Investigator at the research clinic will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the trial. The medical staff has extensive experience from trials with patients requiring invasive portal pressure measurements and intensive surveillance, and there are adequate procedures in place to handle adverse events in the trial subjects. Close monitoring of the subjects during drug infusions, including frequent blood pressure and heart rate measurements, will allow to stop the infusion immediately if needed.

Measurements of hepatic venous pressure gradient (HVPG) is a safe procedure and is used routinely in clinical practice in patients with chronic liver disease. The site is ward at the Hospital Clínic, University of Barcelona, and is located next to both medical and surgical intensive care wards.

It is essential that FE 204205 is administered intravenously, since other routes of administration may cause degradation of the molecule resulting in the generation of the full agonist M1, as shown following subcutaneous administration in healthy subjects. Adequate procedures are in place, according to standard medical practice, to avoid extravasation, i.e. accidental injection or leakage of FE 204205 into the subcutaneous space or perivascular tissues.

The above mentioned risks are addressed in the inclusion and exclusion criteria for the trial and will be closely monitored through safety laboratory evaluations, cardiovascular monitoring including vital signs and ECG, AE reporting, and by safety review committee (SRC) evaluations. The risk posed to the patients participating in the trail is deemed low and ethically justifiable. For more detailed information on FE 204205 please refer to the Investigator´s Brochure (6).

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Objectives

- Safety and tolerability of single intravenous infusions of FE 204205
- Pharmacological effect of FE 204205 on systemic and portal haemodynamics in patients with cirrhotic portal hypertension
- Pharmacokinetics of FE 204205 after intravenous infusion
- Relationship between pharmacokinetics and pharmacodynamics of FE 204205
- Metabolites of FE 204205 in plasma and urine of patients
- Exploratory assessment of effects of FE 204205 on soluble biomarkers and renal function variables

2.2 Endpoints

- Type, frequency and intensity of AEs
- Electrocardiogram (ECG) (intervals, rhythm, and morphology)
- Clinical chemistry, haematology and urinalysis
- Venous blood gases including lactate
- Systemic haemodynamics: Blood pressures and heart rate by non-invasive measurements, pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure (PAP), right atrial pressure (RAP), mean arterial pressure (MAP), and cardiac output (CO) by pulmonary artery catherization. CO, MAP, and RAP will be used for calculation of the systemic vascular resistance (SVR)
- Portal haemodynamics by hepatic vein catheterization: Free hepatic venous pressure (FHVP), wedged hepatic venous pressure (WHVP), and inferior vena cava pressure (IVC). WHVP and the FHVP will be used for calculation of the hepatic venous pressure gradient (HVPG)
- Pharmacokinetics FE 204205: AUC, AUC_t, %extrap AUC, C_{max}, t_{max}, CL, V_z, $t_{½}$, MRT, Ae, and CL_R
- Metabolites in plasma and urine
- Blood and urinary biomarkers: Renin, aldosterone, norepinephrine, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), urine creatinine, urine sodium, and urine osmolality

3 INVESTIGATIONAL PLAN

3.1 Overall Trial Design

3.1.1 Trial Design Diagram

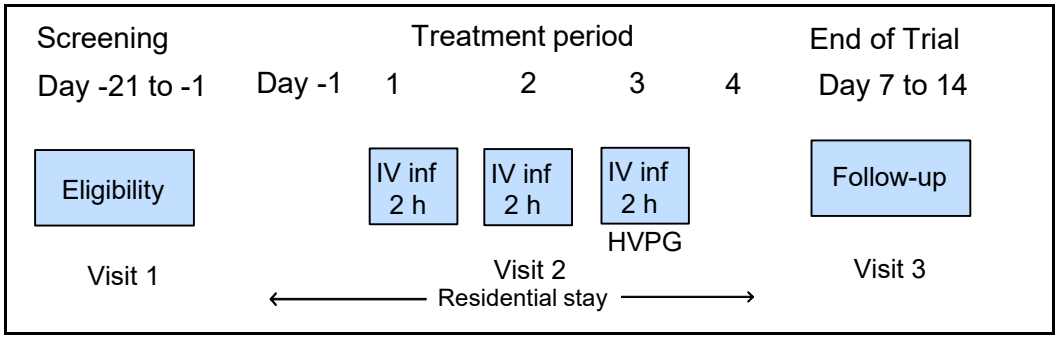

Figure 3-1 Overall study design for each patient in Part 1

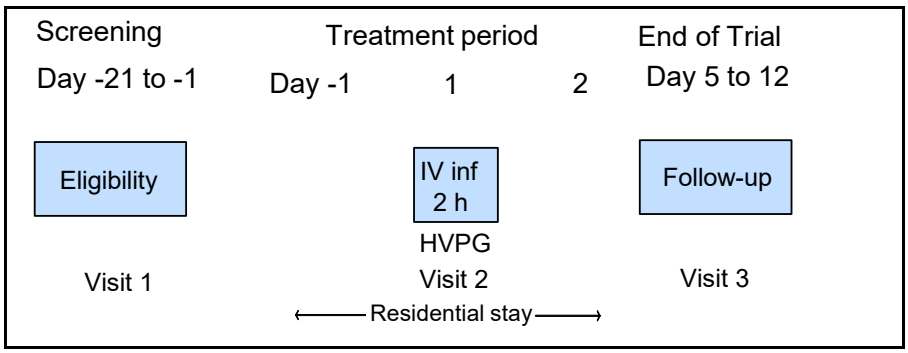

Figure 3-2 Overall study design for each patient in Part 2

3.1.2 Overall Design and Control Methods

This is a trial investigating the safety, tolerability, pharmacokinetics and pharmacodynamics of FE 204205 in cirrhotic patients with portal hypertension. The trial is divided in two parts:

Part 1 is an open-label investigation of safety, tolerability, pharmacokinetics and pharmacodynamics of increasing intravenous doses of FE 204205 and will include 6 patients. Dosing will take place in a staggered fashion, with one patient completing dosing before the next patient will be dosed (i.e. with at least 3 days between administration of the first dose in one patient and the first dose in the next patient). Systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization on Day 3. Each patient will receive three ascending doses of FE 204205, given once daily as 2-hour infusions on three consecutive days. Dose escalation on Days 2 and 3 will only commence provided that the increase in mean arterial

pressure from baseline is <15 mmHg, absolute mean arterial pressure ≤120 mmHg, systolic blood pressure ≤160 mmHg, diastolic blood pressure ≤100 mmHg, and heart rate ≥55 beats per minute at any of the vital signs assessments during the 4 hours after start of infusion on the previous day. If these criteria are not met, the same dose will be administered the next day. If the increase of dose results in non-tolerable effects, the preceding tolerated dose will be reverted to for the remaining administrations in that patient.

For the first 3 patients, the initial treatment given on Day 1 will be a single dose of 0.01 mg FE 204205 followed by planned doses of 0.025 mg FE 204205 on Day 2 and 0.05 mg FE 204205 on Day 3. For the remaining 3 patients, the planned single doses are 0.025 mg FE 204205 on Day 1, 0.05 mg FE 204205 on Day 2 and 0.1 mg FE 204205 on Day 3. After the first 3 patients have completed all Visit 2 assessments, a Safety Review Committee (SRC) will evaluate the preliminary safety, tolerability, and pharmacodynamics data of the FE 204205 and will decide if the dosing regimen can be increased as planned, or if a modified (lower) dose regimen should be applied (see Figure 3-3).

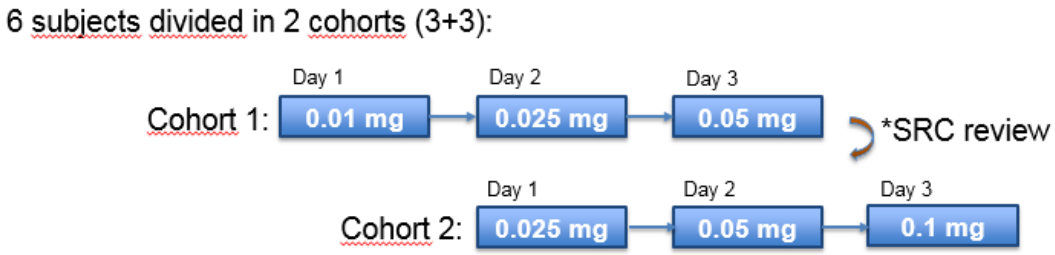

Figure 3-3: Suggested trial design for the 6 patients in Part 1.
*SRC = Safety Review Committee.

HVPG will be the primary pharmacodynamic parameter and assessments will be performed before, during, and immediately after the intravenous administration on Day 3. Patients will be confined to the clinic during the treatment period and will be carefully monitored for adverse events and effects on systemic haemodynamics.

A Safety Review Committee (SRC) will evaluate the safety, tolerability, and pharmacodynamics of the compound after the completion of Part 1 to recommend what dose of FE 204205 to evaluate in Part 2.

Part 2 is a placebo controlled, double-blind, randomised investigation evaluating the effects of a single dose of FE 204205 on portal haemodynamics and will include 16 patients receiving FE 204205 and 4 patients receiving placebo. Each patient will receive a 2-hour intravenous infusion of the maximum tolerated dose of FE 204205 as defined in Part 1, or placebo, on Day 1. Systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization. HVPG will be the primary pharmacodynamic parameter and assessments will be performed before,

during, and immediately after the intravenous administration. Patients will be confined to the clinic during the treatment period and will be carefully monitored for adverse events and effects on systemic haemodynamics.

3.1.3 Trial Schedule

The first subject is expected to be enrolled in Q4 2016 and the last follow-up visit is expected to take place in Q1 2018. The duration of the trial is expected to be approximately 1-1.5 years. The end of trial is defined as the follow-up visit for the last subject.

3.2 Planned Number of Trial Sites and Subjects

The trial will be conducted at one site, University of Barcelona, Hospital clinic, Spain, including patients with cirrhotic portal hypertension, 6 patients in Part 1 completing all Visit 2-assessments, and approx. 20 patients in Part 2, at least 18 of which in Part 2 with HVPG ≥12 mmHg at baseline.

3.3 Interim Analysis

No formal statistical interim analysis is planned.

3.4 Safety Review Committee (SRC)

An SRC, comprising at least one external expert in addition to the Principal Investigator and the Ferring representatives, will evaluate the safety, tolerability and pharmacodynamics of the compound.

Two scheduled meetings are planned: One SRC meeting will be held after the first 3 patients in Part 1 have completed all Visit 2 assessments, where the SRC will recommend whether the doses can be increased as planned or if a modified (lower) dose regimen should be applied. Another SRC meeting will be held after all subjects in Part 1 have completed all Visit 2 assessments in order to determine the dose that will be used in Part 2 of the trial. Furthermore, unscheduled meetings can be demanded anytime for any reasonable cause by the sponsor or the Investigator, e.g. due to any unforeseen adverse events. In such a situation, the SRC will recommend whether the dosing should continue according to the pre-defined dosing schedule, be changed to a different dosing schedule, or whether dosing should be stopped. A working procedure for the SRC will be described prior to study initiation.

3.5 Discussion of Overall Trial Design and Choice of Control Groups

3.5.1 Trial Design

The purpose of Part 1 is to assess safety, tolerability, and pharmacodynamics in the dose interval 0.01-0.1 mg i.v. infusion over 2 hours, and thus determine the maximum tolerated dose within this dose interval. Part 1 is open-label since it comprises only one treatment arm, and it was neither regarded necessary nor ethically justifiable to include placebo treatment. The assessment of systemic and portal hemodynamics by pulmonary artery and hepatic vein catheterization on Day 3 will enable a more precise evaluation of the hemodynamic effect at the maximum tolerated dose, compared to non-invasive vital signs assessments.

Part 2 is aiming at defining the anticipated maximal effects of a single intravenous dose of FE 204205 on portal haemodynamics. Part 2 of the trial is randomised and placebo-controlled in order to correct for the non-treatment related factors that otherwise may have an impact on the evaluation of FE 204205. All subjects will be randomly allocated to active treatment or placebo in a double-blind setting, i.e. the active or placebo treatment will not be revealed to the subjects or the personnel involved in the trial. The randomisation is standard procedure in studies with more than one treatment arm.

3.5.2 Selection of Endpoints

Since this is the first study with FE 204205 in patients with cirrhotic portal hypertension the selected endpoints focus on the evaluation of safety and tolerability of the treatment in general, the pharmacokinetics of FE 204205, and investigation of the pharmacological activity including the pharmacodynamic reponse.

HVPG is the golden standard for evaluation of portal hypertension in patients with cirrhosis. Measurements of HVPG is a safe procedure and it is used routinely in clinical practice in patients with chronic liver disease. The methodology and consistency of the HVPG measurements are crucial in the evaluation of treatment effects. This is ensured by all measurements being performed at one site with the same personnel.

3.5.3 Blinding

Since all subjects in Part 1 receive the same treatment, Part 1 is open-labelled.

Part 2 of the trial is double-blind, keeping all subjects and personnel involved in the trial blinded to the treatment received. Blinding is performed to reduce any bias concerning reporting and interpretation of e.g. AEs. If unblinding is deemed appropriate, the blinding for a patient need not kept once the patient have been treated and all data are entered in the eCRF.

3.5.4 Selection of Doses in the Trial

The selection of doses and dose increments is based on the experiences from two studies in healthy subjects. The doses explored in healthy subjects, ranging between 0.03 to 0.9 mg IV infused over 6 hours, were safe and well tolerated. The IV starting dose of 0.01 mg infused over 2 hours (=0.005 mg/hr) corresponds to the infusion rate applied for the lowest dose level given to healthy subjects (0.03 mg IV infused over 6 hours = 0.005 mg/hr) and is expected to not exceed the exposure in healthy subjects by means of C_{max} (expected C_{max}: approximately 0.3 ng/mL). At this lowest dose level in healthy subjects (0.005 mg/hr IV over 6 hours), no relevant effects on blood pressure were observed. The observed decrease in pulse may have been due to reflex bradycardia and thus may indicate a pharmacodynamic effect. The dose level was safe and well tolerated in healthy subjects, and no gastrointestinal adverse events were reported.

Compared to the first patient (#101) studied in trial 000249, the IV starting dose (introduced with Amendment 2) of 0.01 mg infused over 2 hours (=0.005 mg/hr) corresponds to an infusion rate that is 10 times lower than the one given to patient [redacted] (planned dose of 0.1 mg over 2 hours =0.05

mg/hr). Furthermore, the expected C_{max} after 2 hours of infusion (~0.3 ng/mL) is 3 times below the plasma concentration observed in patient [redacted] (0.95 ng/mL after 30 min).

In Part 1, provided that the dose on the preceding day is regarded safe and tolerable, the subsequent dose can be increased. The planned doses for the first 3 patients are 0.01 mg FE 204205 on Day 1, 0.025 mg FE 204205 on Day 2 and 0.05 mg FE 204205 on Day 3. After the SRC has evaluated the preliminary data on safety, tolerability and pharmacodynamics of FE 204205 from the first 3 patients, the planned doses for the remaining 3 subjects may be increased to maximum 0.025 mg FE 204205 on Day 1, 0.05 mg FE 204205 on Day 2 and 0.1 mg FE 204205 on Day 3 (see protocol section 3.4). Dose escalation in the subject on Days 2 and 3 will only commence provided that the increase in mean arterial pressure from baseline is <15 mmHg, absolute mean arterial pressure ≤120 mmHg, systolic blood pressure ≤160 mmHg, diastolic blood pressure ≤100 mmHg, and heart rate ≥55 beats per minute at all of the vital signs assessments during the 4 hours after start of infusion on the previous day. If these criteria are not met, the same dose will be administered the next day. If the increase of dose results in non-tolerable effects, the preceding tolerated dose will be reverted to for the remaining administration in that subject. The highest planned dose of 0.1 mg infused over 2 hours (=0.05 mg/hr) corresponds to the infusion rate applied for the dose level of 0.3 mg IV infused over 6 hours = 0.05 mg/hr) given to healthy subjects and is expected to not exceed the exposure in healthy subjects by means of C_{max} (expected C_{max}: approximately 3 ng/mL). At this dose level in healthy subjects (0.05 mg/hr IV over 6 hours), the mean arterial pressure increased (max. mean increase of +14 mmHg) and the pulse decreased. The dose level was safe and well tolerated in healthy subjects, as were dose levels up to 3 times higher (highest dose level studied in healthy subjects was 0.9 mg infused over 6 hours = 0.15 mg/hr).

The highest planned dose of 0.1 mg infused over 2 hours (=0.05 mg/hr) that may be reached on Day 3 is the same that was assigned to patient [redacted] on Day 1. However, this dose level will only be reached in a patient in Part 1 who has already tolerated two lower dose levels of FE 204205 on Day 1 and Day 2 and met the dose escalation criteria above.

The chosen dose increments between dose levels (increasing by a factor of 2.5 from the starting dose, then maximum increase of factor 2 for the subsequent doses) are lower and thus more conservative then the ones used in the trials with healthy subjects, where a factor of ~3 was applied for the respective dose range from 0.005 mg/hr to 0.05 mg/hr.

In Part 2 each patient will receive a 2-hour intravenous infusion of the dose of FE 204205 selected by the SRC, or placebo, on Day 1. The SRC will select the dose based on incidence and frequency of adverse events, safety laboratory data, ECG, systemic and portal haemodynamic parameters. The dose selected will be the maximum tolerated dose (MTD) or a lower dose. The MTD is defined as the dose level below the dose where one or more stopping criteria, as confirmed by a second measurement, are met in at least two subjects in Part 1.

3.5.5 Selection and Timing of Dose for Each Subject

The administration of the investigational medicinal product (IMP) will commence in the morning during fasting conditions.

3.5.6 Selection of the Trial Population

Subjects with cirrhotic portal hypertension and anticipated HVPG ≥12 mmHg, are chosen as trial population since this is the target population. Measures will be taken to reach a balanced ratio between genders (i.e. at least ¼ of each gender) in the trial.

3.5.7 Withdrawal Criteria

The subject should be withdrawn if any signs of serious, or unexpected and unacceptable treatment emergent AEs occur, and may be withdrawn if the inclusion or exclusion criteria are violated during the conduct of the study. The subjects have the right to withdraw at any time for any reason without justification. In addition, a number of stopping criteria are defined, related to cardiovascular parameters (see Section 4.5).

3.5.8 Follow-up Procedures

After the end-of-trial, the subject is referred to standard treatment according to medical need under the responsibility of the treating physician.

4 SELECTION OF TRIAL POPULATION

4.1 Trial Population

Subjects, 18-65 years of age, with verified cirrhotic portal hypertension and anticipated HVPG ≥12 mmHg.

4.1.1 Inclusion Criteria

1. Signed written informed consent
2. Male or female patient, 18-65 years of age with confirmed evidence of cirrhosis
3. From medical history anticipated HVPG ≥12 mmHg
4. Negative pregnancy test at Screening and on Day -1
5. Agrees to use an adequate method of contraception during the study until the follow-up visit, if not abstinent. Adequate methods of contraception include condom with or without spermicidal gel (adequate only for male participants), intrauterine device, surgical sterilisation, vasectomy, oral contraceptive pill associated with inhibition of ovulation, depot progesterone injections
6. Negative urine drug screen and alcohol urine test at Screening and on Day -1

4.1.2 Exclusion Criteria

1. Patients with co-existing disease including but not restricted to significant organ failure, decompensated cirrhosis with a Child Pugh score >12 or requirement for organ support, and acute pancreatitis
2. Type 1 hepatorenal syndrome
3. Severe hyponatremia (serum sodium concentration <125 mEq/L)
4. Acute-on-chronic liver failure
5. Hepatic encephalopathy ≥grade 2
6. Hepatocellular carcinoma outside the Milan criteria (<5 cm for a single lesion or less than 3 lesions with the largest measuring ≤3 cm)
7. Bacterial infection within 7 days prior to dosing
8. Gastrointestinal bleeding within 6 weeks prior to dosing.
9. Severe renal impairment i.e. estimated creatinine clearance < 30mL/min calculated according to the Modification of Diet in Renal Disease (MDRD) study equation
10. History of underlying chronic heart disease, including but not restricted to heart failure NYHA class III or IV, angina pectoris, or myocardial infarction
11. Diagnosed ongoing hypertension
12. Symptomatic peripheral vascular disease including Raynaud's syndrome
13. Diagnosed chronic obstructive pulmonary disease (COPD)
14. Severe grade 3 anemia, i.e. Hb < 8.0 g/dL (< 4.9 mmol/L)

15. Use of vasopressin or terlipressin within 7 days prior to dosing
16. Permanent use of beta-blocker or a long-acting so called nitro-dilator, or if temporary use within 7 days prior to dosing
17. Use of treatments for hepatitis B or C virus within 6 months of randomisation or anticipated use during the trial period
18. Positive serology for human immunodeficiency virus (HIV)-1 or HIV-2
19. Women being pregnant or breastfeeding at screening and Day -1
20. Hypersensitivity towards any component of FE 204205 formulation
21. Sensitivity to contrast media
22. Intake of an investigational medicinal product within the last 8 weeks preceding screening or longer if judged by the investigator to possibly influence the outcome of the current study
23. Body mass index (BMI) <18 or >35 kg/m^2
24. Abuse of alcohol or drugs within 6 months prior to screening
25. Mental incapacity or language barrier precluding adequate understanding or co-operation
26. Previously included in this trial
27. Considered by the investigator to be unsuitable to participate in the study for any other reason

4.2 Method of Assigning Subjects to Treatment Groups

4.2.1 Recruitment

Patients with cirrhotic portal hypertension will be recruited among the patients enlisted for treatment at hospital clinics in the Barcelona area. However, all treatments will be conducted at the University of Barcelona, Hospital Clinic.

4.2.2 Randomisation

All subjects screened will be assigned a screening number. A subject number will be allotted to the subject according to a sequential subject number list in the order at which the subjects are found eligible. In Part 1, the subjects are not randomised. In Part 2, all subjects will be randomised to active treatment or placebo according to a computer-generated randomisation list provided by Global Biometrics, Ferring Pharmaceuticals A/S. Randomisation will be carried out on Day 1 before administration of IMP.

4.3 Restrictions

4.3.1 Prior and Concomitant Therapies

Concomitant medication with vasopressor substances, beta-blockers, or nitro-dilators is not allowed. Other concomitant medication may be administered at the discretion of the Investigator. Any concomitant medication will be recorded in the eCRF, together with the main reason for its prescription.

4.3.2 Prohibited Therapy

Treatment with vasopressor substances (e.g. vasopressin, terlipressin, or catecholamines) or use of beta-blockers or a long-acting so called nitro-dilator, is not allowed.

4.3.3 Other Restrictions

Subjects should abstain from drinking alcoholic beverages for 72 hours before the screening visit and all subsequent visits to the clinical investigation unit. In addition, subjects should abstain from beverages containing caffeine and alcoholic beverages during the residential stay in both trial parts.

Intake of products containing poppy seeds (e.g., poppy cake) should be avoided for a period of at least 7 days before screening and admission to the clinic in both trial parts in order to avoid analytical interference with the drug screen for opiates.

The subjects should abstain from strenuous physical activity that is not within the subject's normal weekly routine for 48 hours prior to screening and from 48 hours prior to admission until the last safety examination in both trial parts.

Subjects must not participate in another clinical trial during the course of the present trial.

4.4 Withdrawal Criteria

The Investigator has the right and obligation to withdraw a subject should any signs of serious, or unexpected and unacceptable treatment emergent AEs occur. Furthermore, a subject may be withdrawn from the study if the inclusion or exclusion criteria are violated during the conduct of the study.

Both the Investigator (with regard to his/her participation) and Ferring, reserves the right to discontinue the study at any time for safety reasons, such as signs of unacceptable adverse reactions, or mortality, or any other reasons jeopardising the justification of the study. Such a termination will be implemented in a time frame that is compatible with the subject's well-being. If the study is prematurely terminated or suspended, the investigator should promptly inform the subjects and assure appropriate follow-up and treatment if required. Ferring Pharmaceuticals A/S will notify the Regulatory Authorities of any plans to terminate the study, and the Investigator will notify the Independent Ethics Committee (IEC).

The subjects have the right to withdraw from the trial at any time for any reason, without the need to justify their decision. For any discontinuation, the Investigator will obtain all the required details and document the date of the premature termination and the main reason in the eCRF. If possible, follow-up assessments should be performed for subjects who are withdrawn after start of infusion.

4.5 Stopping Criteria

The infusion will be interrupted and may be adjusted in the subject if any of the below stopping criteria occur, and halted for evaluation by the SRC when one or more criteria, as confirmed by a second measurement, are met in at least two subjects receiving the same treatment.

- Systolic blood pressure increased by 50% or more from the baseline and reaching >160 mmHg
- Systolic blood pressure 180 mmHg or higher
- Diastolic blood pressure 105 mmHg or higher
- Mean arterial pressure 130 mmHg or higher
- Heart rate 45 beats per minute or lower
- Signs or symptoms of mesenteric or peripheral ischemia, or hypertensive crises.

4.6 Subject replacement

Subjects withdrawn prematurely in Part 1 will be replaced in order to achieve 6 subjects that complete all Visit 2-assessments.

5 TREATMENTS

A separate instruction will cover all procedures regarding handling, dilution, and administration of the IMPs. The manual will be provided to the clinical investigational unit before the start of the study.

5.1 Treatments Administered

5.1.1 Investigational Medicinal Product

The IMPs in this trial are FE 204205 10 mg/mL and placebo (commercially available 0.9% sodium chloride).

5.1.1.1 Part 1

In Part 1, each subject will receive three ascending doses of FE 204205, given once daily as 2-hour intravenous infusions on three consecutive days.

Table 5-1 Planned dose regimen of FE 204205, Part 1

(Doses to be infused i.v. over 2 hours and respective infusion rates)

Treatment Day	Dose (Patients 1-3)	Dose (Patients 4-6)
Day 1	0.01 mg (0.005 mg/hr)	0.025 mg (0.0125 mg/hr)
Day 2	0.025 mg (0.0125 mg/hr)	0.05 mg (0.025 mg/hr)
Day 3	0.05 mg (0.025 mg/hr)	0.1 mg (0.05 mg/hr)

Planned doses and infusion rates are listed in Table 5-1. After the first 3 patients have completed all Visit 2 assessments, the SRC will evaluate the preliminary safety, tolerability, and pharmacodynamics data of the FE 204205 and will decide if the dosing regimen can be increased as planned, or if a modified (lower) dose regimen should be applied. A modified dosing regimen may be chosen by the SRC (also in unscheduled meetings, if deemed necessary by the Investigator or Sponsor) as long as the modified planned infusion rates do not exceed the infusion rates given in Table 5-1, and as long as the modified increments between the dosing days (from Day 1 to Day 2 and from Day 2 to Day 3) do not exceed factor 2.

5.1.1.2 Part 2

In Part 2, each subject will receive a single 2-hour intravenous infusion of FE 204205 or placebo.

The SRC will evaluate the safety and tolerability of the compound after the completion of Part 1 to determine the maximum tolerated dose within the administered dose range of FE 204205, and recommend which dose to be investigated in Part 2.

5.1.2 Non-Investigational Medicinal Product

Commercially available 0.9% sodium chloride to be used for dilution of IMP will be supplied by the Sponsor. Commercially available midazolam for sedation, mepivacaine for local anaesthesia, and the contrast medium iodine will be provided by the site.

5.2 Characteristics and Source of Supply

The IMPs are provided by Ferring Pharmaceuticals A/S and will be handled according to the principles of Good Manufacturing Practice (GMP). Ferring Pharmaceuticals A/S will provide the pharmacy at University of Barcelona, Hospital Clinic with IMP and non-IMP in amounts sufficient for the trial.

5.2.1 Investigational Medicinal Products

The stock solution of FE 204205 is detailed in (Table 5-2). FE 204205 will be diluted with 0.9% sodium chloride to the desired concentrations. Commercially available 0.9% sodium chloride will be used as placebo.

Table 5-2 Composition of IMP

	FE 204205
FE 204205	10 mg/mL
Sodium acetate buffer	10 mM, pH 4.5
Mannitol	44.3 mg/mL
Sodium chloride	---

5.3 Packaging and Labelling

Packaging and labelling of the medicinal products will be performed under the responsibility of the IMP department at Ferring Pharmaceuticals A/S in accordance with GMP and national regulatory requirements.

The IMP will be labelled according to Annex 13, EudraLex Volume 4 and national regulatory requirements. The label of the IMP will contain one self-adhesive tear-off portion to be affixed to the Subject dispensing log, or similar, maintained at the trial site. The IMPs will be labelled with a unique IMP number.

5.4 Conditions for Storage and Use

Storage condition is stated at the labels of the IMP and non-investigational medicinal product (non-IMP). The Investigator will ensure that the IMPs and non-IMP will be stored according to storage condition at the label, in a secure location with controlled access. The storage compartments shall be monitored regularly and the temperature shall be documented. Deviations in storage temperature must be reported to Sponsor without delay and the IMP must not be used until acceptance from the Sponsor is received.

5.5 Blinding / Unblinding

5.5.1 Blinding

Part 1 of the trial is open-labelled. In Part 2, all subjects, the Investigator, and all staff involved in the conduct of the trial will be blinded to the treatment received. The trial monitor will not be blinded, and no specific precautions will be taken to keep the pharmacist or personnel running the analyses of FE 204205 blinded.

It is the responsibility of the pharmacy to ensure that the syringes used for infusion in Part 2 are identical and non-distinguishable.

The pharmacy will receive the computer-generated randomisation list from Global Biometrics, Ferring Pharmaceuticals A/S.

5.5.2 Unblinding of Individual Subject Treatment

An emergency decoding system will be available to the Investigator at University of Barcelona Hospital Clinic and to designated persons at Ferring Pharmaceuticals for each subject. Breaking of the blind for individual subjects in emergency situations is only permitted in case of a serious, unexpected, or other important AE, when the knowledge of the trial medication in question is required for therapeutic decisions and for the management of the subject. As far as the emergency permits, the need to break the blind will be agreed by the Investigator and Ferring. If the blinding is broken it must be documented by whom, reason and the date of unblinding.

If it is necessary to unblind an individual subject's treatment for the purposes of expedited reporting to the authorities and/or IEC, only those individuals within Ferring whose responsibility it is to report this information will know the identity of the trial medication. Every attempt will be made to try to keep all other trial and site personnel blinded throughout the course of the trial.

Information on whether the blind has been broken for any subjects must be collected before the database is declared clean and is released to the statistician.

If unblinding is deemed appropriate, the blinding for a patient need not kept once the patient have been treated and all data are entered in the eCRF.

5.6 Treatment Compliance

5.6.1 Dispensing and Accountability

The Investigator will maintain a drug accountability logs or equivalent detailing the dates and quantities of study medication dispensed to, and used by, each subject, as well as the batch numbers. The Monitor will verify the drug accountability during the trial.

5.6.2 Assessment of Compliance

The study medication will be administered by authorised staff at the clinical investigation unit. Compliance is assessed by recording the volume infused.

5.7 Auxiliary Supplies

University of Barcelona, Hospital Clinic, will use auxiliary supplies as instructed by Ferring Pharmaceuticals A/S. The materials will be provided from commercial lots and supplied by the site. No modification to the commercial state will be made.

5.8 Return and Destruction of Medicinal Products and Auxiliary Supplies

Used IMP vials, sodium chloride infusion bags, and auxiliary supplies used for dilution and infusion will be destroyed at site according to local regulations and standard procedures at the site after the drug accountability has been verified by the Monitor and signed off by the Sponsor.

Documentation on destruction will be forwarded to the Sponsor.

Any unused IMP and sodium chloride will be returned for destruction, as instructed by IMP Department, Ferring Pharmaceuticals A/S, and in accordance with local requirements, after the drug accountability has been finalised, verified by the Monitor, signed off by the Investigator, and approved by the Sponsor.

6 TRIAL PROCEDURES

For each subject, the duration of the treatment period, including the screening period and the follow-up visit will not exceed 5 weeks. Assessments of concomitant medication and AEs will be performed throughout both study periods.

FE 204205 or placebo will be administered as a continuous 2-hour intravenous infusion starting in the morning. The infusion may be paused or slowed down if tolerability issues arise. The patient will be monitored during the infusion, including assessment of pulse rate and blood pressure.

6.1 Part 1

Each patient will receive three ascending doses of FE 204205, given once daily as 2-hour infusions on three consecutive days.

Visit 1 (Screening, Day -21 to Day -1)

At the screening visit each subject will be informed about the study and after signing the Informed Consent form assessments for eligibility will commence. The eligibility evaluation includes adherence to inclusion and exclusion criteria, collection of concomitant medication, demographic data and medical history, physical examination, urine drug screen, alcohol urine test, assessment of vital signs and 12-lead ECG, blood sampling for serology, pregnancy test, and clinical chemistry, haematology, and urine sampling for urinalysis (i.e. clinical laboratory parameters).

Visit 2 (Residential stay, Day -1 to Day 4)

Subjects found eligible will be asked to return for a treatment visit (residential stay). At the arrival to the clinic on the day before the first administration day (Day -1), a general eligibility check (inclusion/exclusion criteria, medical history, urine drug screen, alcohol urine test, assessment of vital signs and 12-lead ECG, pregnancy test, blood and urine sampling for clinical laboratory parameters) will be performed. A baseline assessment of venous blood gases including lactate will also be performed.

The next day (Day 1) subjects will be dosed with a 2-hour continuous intravenous infusion of FE 204205 (for planned doses see Protocol Section 5.1.1.1). Before administration of FE 204205 the subjects body weight will be assessed. Safety assessments including sampling for clinical laboratory parameters, assessment of vital signs, 12-lead ECG, venous blood gases including lactate, and urinary output will be performed at predetermined time-points (Table 6-1). Blood samples for pharmacokinetic determination, metabolites, biomarkers, and samples for urinary biomarkers and excretion of FE 204205 and metabolites will be collected at predetermined time-points (Table 6-1).

On Day 2 and Day 3 the subject will be administered ascending doses of FE 204205 through 2-hour continuous infusions. Before each administration of FE 204205 the subjects body weight will be assessed. Safety assessments including sampling for clinical laboratory parameters, assessment of vital signs, 12-lead ECG, venous blood gases including lactate, and urinary output will be

performed at predetermined time-points (Table 6-1). Blood samples for pharmacokinetic determination, metabolites, biomarkers, and samples for urinary biomarkers and excretion of FE 204205 and metabolites will be collected at predetermined time-points (Table 6-1). On Day 3, in addition to the same assessments performed on Day 2, systemic and portal haemodynamics will be assessed by pulmonary artery and hepatic vein catheterization with measurements performed at pre-determined time-points before, during and immediately after the intravenous infusion (Table 6-1).

HVPG is the golden standard for evaluation of portal hypertension in patients with cirrhosis. It is a moderately invasive technique and catherization of the patients will be carried out under mild sedation with midazolam and local anaesthesia. A balloon-tipped catheter is placed in the right or middle hepatic vein and the FHVP and WHVP are measured, the latter is when the vein is occulated by the balloon.

After an overnight fast, the patients will be transferred to the Hepatic Haemodynamic Laboratory. Under mild sedation and local anesthesia, a venous catheter introducer will be placed using ultrasonographic guidance in the right jugular vein by the Seldinger technique. Under fluoroscopic control, a Swan-Ganz catheter will be advanced to the pulmonary artery for measurement of baseline (pre-dose) cardiopulmonary pressures and cardiac output by the thermal dilution method. After withdrawal of the Swan-Ganz catheter, a balloon-tipped catheter will be guided using fluoroscopy into the inferior vena cava and subsequently to the main right or middle hepatic vein for measurements of baseline (predose) FHVP, WHVP, and IVC. The HVPG results from the difference between WHVP (occluded) and FHVP. The adequacy of occlusion will be checked by gentle injection of a small amount of radiologic contrast medium after balloon inflation. All measurements will be performed at least in duplicate (the average will be used for the endpoint) and permanent tracings will be obtained with a multichannel recorder and adequately calibrated transducers. The catheter will be kept in the hepatic vein for assessments of FHVP and WHVP at 1 and 2 hours after start of infusion of IMP. At withdrawal of the catheter (at 2 hours after start of infusion) IVC will be reexamined. Immediately after the removal of the balloon-tipped catheter, cardiopulmonary pressures and cardiac output will be reassessed (at 2 hours after start of infusion) using a Swan-Ganz catheter advanced to the pulmonary artery under fluoroscopic control. During the haemodynamic study all patients will be subjected to noninvasive monitoring by continuous display of heart rate, pulse oximetry, and respiratory rate, along with frequent blood pressure measurements.

The subjects will be discharged from the clinic after the post-dose assessments (clinical laboratory parameters, vital signs, 12-lead ECG, body weight) on Day 4 (Table 6-1) have been performed.

Visit 3 (Follow-up, Day 7 to Day 14)

A follow-up visit will be performed 4-11 days after the last administration, and will be considered end of study. At this visit a physical examination, assessment of vital signs and 12-lead ECG

recording will be performed, pregnancy test, and samples for clinical laboratory parameters will be collected.

Table 6-1 Study Flow Chart, Part 1

Assessment	**Screening**	**Treatment Period**					**End of trial**
Visit No.	Visit 1	Visit 2					Visit 3
Procedures / Days	-21 to -1	Day -1	Day 1	Day 2	Day 3	Day 4	Days 7-14
Informed consent	X						
Inclusion and exclusion criteria	X	X					
Demographics	X						
Physical examination	X						X
Medical history	X	X					
Clinical chemistry, haematology, urinalysis	X	X	X[1]	X[1]	X[1]	X[1]	X
Serology/virology	X						
Urine drug screen	X	X					
Alcohol urine test	X	X					
Pregnancy test (serum)	X	X					X
12-lead ECG	X	X	X[2]	X[2]	X[2]	X[2]	X
Vital signs (non-invasive)	X	X	X[3]	X[3]	X[3]	X[3]	X
Adverse events	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X
Venous blood gas including lactate		X	X[4]	X[4]	X[4]		
Administration of FE 204205			X	X	X		
Blood samples for PK and metabolites			X[5]	X[5]	X[5]		
Hepatic vein and pulmonary artery catheterization					X[8]		
Blood biomarkers			X[6]	X[6]	X[6]		
Urine sampling for urinary output, urinary biomarkers and excretion of FE 204205 and metabolites			X[7]	X[7]	X[7]		
Body weight			X[9]	X[9]	X[9]	X[9]	

[1] Blood samples for safety laboratory evaluations will be collected 6 hours after start of infusion on Days 1-3, and in the morning on Day 4.

[2] ECG will be collected pre-dose and at 1, 2, 3, and 6 hours after the start of the infusion on Days 1-3, and in the morning on Day 4.

[3] Vital signs comprising systolic and diastolic arterial blood pressure (including calculation of mean arterial pressure) and pulse will be assessed pre-dose, every 5 min until 30 min after start of infusion, then every 10 min until 1 hour after start of infusion, then every 15 min until 2.5 hours after start of infusion and therafter 3, 4, and 6 hours after start of infusion on Days 1-3, and in the morning on Day 4. Temperature only pre-dose and 6 hours after after start of infusion on Days 1-3.

[4] Venous blood gas including lactate will be collected 3 hours after the start of the infusion on Days 1-3.

[5] Blood samples for measurement of plasma concentration of FE 204205 and metabolites will be collected pre-dose and at 0.5, 1, 2, 3 and 4 hours after the start of the infusion on Days 1-3.

[6] Blood samples for measurement of biomarkers will be collected pre-dose and at 3 hours after the start of the infusion on Days 1-3.

[7] Urinary output, urinary biomarkers and excretion of FE 204205 and metabolites will measured pre-dose and after each spontaneous void up to 8 hours after start of infusion on Days 1-3.

[8] Measurement of portal haemodynamics by hepatic vein catheterization will be performed pre-dose and at 1 and 2 hours after start of the infusion on Day 3. Pulmonary artery catheterization will be performed pre-dose and at 2 hours after start of the infusion on Day 3.

[9]Body weight will be assessed pre-dose on Days 1-3, and in the morning on Day 4.

6.2 Part 2

Each patient will receive a 2-hour intravenous infusion of FE 204205 or placebo on Day 1.

Visit 1 (Screening, Day -21 to Day -1)

At the screening visit each subject will be informed about the study and after signing the Informed Consent form assessments for eligibility will commence. The eligibility evaluation includes adherence to inclusion and exclusion criteria, collection of concomitant medication, demographic data and medical history, physical examination, urine drug screen, alcohol urine test, assessment of vital signs and 12-lead ECG, blood sampling for serology, pregnancy test, and clinical chemistry, haematology, and urine sampling for urinalysis (i.e. clinical laboratory parameters).

Visit 2 (Residential stay, Day -1 to Day 2)

Subjects found eligible will be asked to return for a treatment visit (residential stay). At the arrival to the clinic on the day before the administration day (Day -1), a general eligibility check (inclusion/exclusion criteria, medical history, urine drug screen, alcohol urine test, assessment of vital signs and 12-lead ECG, pregnancy test, blood and urine sampling for clinical laboratory parameters) will be performed. A baseline assessment of venous blood gases including lactate will also be performed.

The next day (Day 1) subjects will be randomised to 2-hour continuous infusion of either FE 204205 or placebo. Before administration of either FE 204205 or placebo the subjects body weight will be assessed. Safety assessments including sampling of clinical laboratory parameters, assessment for vital signs, 12-lead ECG, venous blood gases including lactate, and urinary output will be performed at predetermined time-points (Table 6-2). Systemic and portal heamodynamics will be assessed by pulmonary artery and hepatic vein catheterization (for details see Part 1 Visit 2) at the predetermined time-points specified in Table 6-2. Blood samples for pharmacokinetic determination, metabolites, metabolite pattern, biomarkers, and samples for urinary biomarkers and excretion of FE 204205 and metabolites will be collected at predetermined time-points (Table 6-2).

The subjects will be discharged from the clinic after the post-dose assessments (clinical laboratory parameters, vital signs, 12-lead ECG, body weight) on Day 2 have been performed.

Visit 3 (Follow-up, Day 5 to Day 12)

A follow-up visit will be performed 4-11 days after the administration, and will be considered end of study. At this visit a physical examination, assessment of vital signs and 12-lead ECG recording will be performed, pregnancy test, and samples for clinical laboratory parameters will be collected (Table 6-2).

Table 6-2 Study Flow Chart, Part 2

Assessment	**Screening**	**Treatment Period**			**End of trial**
Visit No.	Visit 1	Visit 2			Visit 3
Procedures / Days	-21 to -1	Day -1	Day 1	Day 2	Days 5-12
Informed consent	X				
Inclusion and exclusion criteria	X	X			
Randomisation			X		
Demographics	X				
Physical examination	X				X
Medical history	X	X			
Clinical chemistry, haematology, urinalysis	X	X	X[1]	X[1]	X
Serology/virology	X				
Urine drug screen	X	X			
Alcohol urine test	X	X			
Pregnancy test (serum)	X	X			X
12-lead ECG	X	X	X[2]	X[2]	X
Vital signs (non-invasive)	X	X	X[3]	X[3]	X
Adverse events	X	X	X	X	X
Concomitant medication	X	X	X	X	X
Venous blood gas including lactate		X	X[4]		
Administration of IMP			X		
Blood samples for PK and metabolites			X[5]		
Blood samples for metabolite pattern			X[9]		
Hepatic vein and pulmonary artery catheterization			X[6]		
Blood biomarkers			X[7]		
Urine sampling for urinary output, urinary biomarkers and excretion of FE 204205 and metabolites			X[8]		
Body weight			X[10]	X[10]	

[1] Blood samples for safety laboratory evaluations will be collected 6 hours after start of infusion on Day 1, and in the morning on Day 2.

[2] ECG will be collected pre-dose and at 1, 2, 3, and 6 hours after the start of the infusion on Day 1, and in the morning on Day 2.

[3] Vital signs comprising systolic and diastolic arterial blood pressure (including calculation of mean arterial pressure) and pulse will be assessed pre-dose, every 5 min until 30 min after start of infusion, then every 10 min until 1 hour after start of infusion, then every 15 min until 2.5 hours after start of infusion and therafter 3, 4, and 6 hours after start of infusion on Day 1, and in the morning on Day 2. Temperature only pre-dose and 6 hours after start of infusion on Day 1.

[4] Venous blood gas including lactate will be collected 3 hours after the start of the infusion on Day 1.

[5] Blood samples for measurement of plasma concentration of FE 204205 and metabolites will be collected pre-dose and at 0.5, 1, 2, 2.5, 3, 4 and 5 and after the start of the infusion on Day 1.

[6] Measurement of portal haemodynamics by hepatic vein catheterization will be performed pre-dose and at 1 and 2 hours after start of the infusion on Day 1. Pulmonary artery catheterization will be performed pre-dose and at 2 hours after start of infusion.

[7] Blood samples for measurement of biomarkers will be collected pre-dose and at 3 hours after the start of the infusion on Day 1.

[8] Urinary output, urinary biomarkers and excretion of FE 204205 and metabolites will measured pre-dose and after each spontaneous void up to 8 hours after start of infusion.

[9]Blood samples for metabolite pattern will be collected pre-dose and at 3 hours after the start of infuion on Day 1
[10]Body weight will be assessed pre-dose on Day 1 and and in the morning on Day 2.

7 TRIAL ASSESSMENTS

7.1 Assessments Related to Endpoints

Actual sampling times for assessments will be recorded in the eCRF.
In the event that the infusion is stopped prior to two hours after start, all subsequent assessments related to endpoints should be performed as if the infusion stopped at 2 hours, i.e. PK sampling in Part 1 at infusion stop and 1 and 2 hours after stop, vital signs in Part 1 at infusion stop and 0.25, 0.5, 1, 2, and 4 hours after stop, etc.

7.1.1 Adverse Events

AEs will be recorded during the trial period from the obtaining the informed consent to the follow-up visit for both Part 1 and Part 2. For further information on definitions and reporting of AEs and SAEs, see Section 8.

7.1.2 Vital Signs

Vital signs comprising systolic and diastolic arterial blood pressure (including calculation of mean arterial pressure) and pulse will be assessed at screening, on Day -1, pre-dose, every 5 min until 30 min after start of infusion, then every 10 min until 1 hour after start of infusion, then every 15 min until 2.5 hours after start of infusion and thereafter 3, 4, and 6 hours after start of infusion on Days 1-3, and in the morning of Day 4 as well as at the follow-up visit in Part 1. Body temperature will be measured pre-dose and 6 hours after start of infusion on Days 1-3 in Part 1.

In Part 2, vital signs will be assessed at screening, on Day -1, pre-dose, every 5 min until 30 min after start of infusion, then every 10 min until 1 hour after start of infusion, then every 15 min until 2.5 hours after start of infusion and thereafter 3, 4, and 6 hours after start of infusion on Day 1, in the morning of Day 2, and at the follow-up visit. Body temperature will be measured pre-dose and 6 hours after start of infusion on Day 1.

Systolic and diastolic blood pressure will be measured after the subject has been in supine position for at least 5 minutes. All recordings will be performed using validated standard equipment. Clinically significant abnormal findings will be reported as AEs. Additional vital signs assessments may be collected by the Investigator for safety reasons.

7.1.3 ECG

12-lead ECGs will be recorded at screening, Day -1, pre-dose and 1, 2, 3, and 6 hours after start of infusion of FE 204205 on Days 1-3, in the morning of Day 4, and at the follow-up visit in Part 1.

In Part 2, 12-lead ECGs will be recorded at screening, Day -1, pre-dose and 1, 2, 3, and 6 hours after start of infusion on Day 1, in the morning of Day 2, and at the follow-up visit.

The ECGs will be recorded with a validated ECG device. The parameters HR, PR interval, RR, QRS, QT, and QTc(F) (i.e. QT correction according to the Fridericia formula $QTcF = QT/RR^{0.33}$) will be assessed. ECG recordings will capture at least four QRS complexes, i.e. 3 evaluable RR

intervals. The Investigator or a designate will evaluate whether the ECG is normal or abnormal and whether it is clinically significant, if abnormal. Any occurrence of de- or re-polarisation disorders, arrhythmic disorders or other abnormalities will be assessed and any changes compared to the pre-medication record will be commented. Clinically significant abnormal findings will be reported as AEs. Additional ECGs may be collected by the Investigator for safety reasons.

7.1.4 Clinical Laboratory Variables

Clinical chemistry, and haematology

In Part 1, blood samples for safety laboratory evaluations of clinical chemistry and haematology parameters (Table 7-1) will be collected at screening, on Day -1, 6 hours after start of intravenous infusion of FE 204205 on Days 1-3, in the morning of Day 4, and at the follow-up visit.

In Part 2, blood samples for safety laboratory evaluations of clinical chemistry and haematology parameters (Table 7-1) will be collected at screening, on Day -1, 6 hours after start of intravenous infusion on Day 1, in the morning of Day 2, and at the follow-up visit.

The actual sampling times will be recorded. The clinical chemistry, and haematology analyses will be performed locally. Clinically significant abnormal findings will be reported as AEs.

Urinalysis

Urine samples for safety laboratory evaluation of urinalysis parameters (Table 7-1) will in Part 1 be collected at screening, on Day -1, 6 hours after the start of the intravenous infusion of FE 204205 on Days 1 to 3, in the morning of Day 4, and at follow-up.

In Part 2, urinalysis samples will be collected at screening, on Day -1, 6 hours after the start of the intravenous infusion on Day 1, in the morning of Day 2, and at follow-up.

Urinalysis will be performed locally from a sample of mid-stream urine by means of a dip-stick test. In case any result of the dipstick is abnormal, a new urine test will be performed. In case any result of the dipstick is abnormal and clinically significant, a new urine test will be performed. If the abnormal result is confirmed, further examination may be initiated at the discretion of the Investigator. Clinically significant abnormal findings will be reported as AEs.

Table 7-1 Safety Laboratory Parameters

Clinical Chemistry	Haematology	Urinalysis
Alanine aminotransferase	Haematocrit	Protein
Albumin	Haemoglobin	Glucose
Alkaline phosphatise	Mean cellular volume (MCV)	Bilirubin
Aspartate aminotransferase	Mean corpuscular haemoglobin content (MCH)	pH
Glucose	Mean corpuscular haemoglobin concentration (MCHC)	Nitrite
Calcium	Platelet count	Ketone
Chloride	Red blood cell count	Urobilinogen
Cholesterol	Reticulocytes	Blood
C-reactive protein	White blood cell count with differential count	Leukocytes
Creatinine	Neutrophils, lymphocytes, eosinophils, and basophils	Specific gravity
Gamma-glutamyltransferase	Monocytes, large unclassified cells	
Phosphate		
Potassium		
Sodium		
Total bilirubin		
Triglycerides		
Troponin I		
Urea (blood urea nitrogen)		

7.1.5 Venous Blood Gases including Lactate

Samples for analysis of blood gases (O_2 and CO_2) and lactate will be collected on Day -1, 3 hours after start of infusion on Days 1-3 in Part 1, and on Day -1 and 3 hours after start of infusion on Day 1 in Part 2.

Additional samples may be collected at the discretion of the Investigator.

7.1.6 Urinary Output, Urinary Biomarkers and Excretion of FE 204205 and Metabolites

In Part 1, the urinary output will be recorded pre-dose and after each spontaneous void up to 8 hours after start of infusion on Days 1-3. The urine collected will be analysed for biomarkers (urine creatinine, urine sodium, and urine osmolality), and amount of FE 204025 and its metabolites (see Section 7.1.8).

In Part 2, the urinary output will be recorded pre-dose and after each spontaneous void up to 8 hours after start of infusion on Day 1. The urine collected will be analysed for biomarkers (urine creatinine, urine sodium, and urine osmolality), and amount of FE 204025 and its metabolites (see Section 7.1.8).

7.1.7 Blood Hormones/Biomarkers

Blood samples for measurement of biomarkers i.e. reninand aldosterone will be collected pre-dose and at 3 hours after start of each infusion for Part 1 and renin, aldosterone, norephinephtine, atrial natriuretic peptide, and brain natriuretic peptide will be collected pre-dose and at 3 hours after start of the infusion for Part 2.

The analysis of the biomarkers will be performed at the University of Barcelona, Hospital Clinic.

7.1.8 Metabolites in Plasma and Urine

Metabolites will be analysed in blood collected pre-dose, and at 1, 2, 3, 4, and 6 hours after the start of infusion on Days 1-3 in trial Part 1. In Part 2, blood for analysis of metabolites will be collected pre-dose, 0.5, 1, 2, 3, 4, 5 and at 6 hours after the start of infusion on Day 1.

Blood samples for metabolite pattern will be collected pre-dose and at 3 hours after start of infusion on Day 1 in trial Part 2.

Metabolite analysis will be performed for urine samples taken pre-dose and after each spontaneous void up to 8 hours after start of infusion on Days 1-3 (Part 1) and Day 1 (Part 2).

The analysis of metabolites will be performed under the responsibility of the Bioanalysis Department, Ferring Pharmaceuticals A/S, by means of liquid chromatography coupled mass spectrometry (LC-MS). The analysis will be performed within EU.

7.1.9 Pharmacokinetics

Blood samples for measurement of plasma concentration after the intravenous administration of FE 204205 will be collected pre-dose, and at 0.5, 1, 2, 3, and 4 hours after the start of infusion on Days 1-3 in trial Part 1. The actual sampling time will be recorded in the eCRF.

In Part 2, samples for FE 204205 plasma concentration measurements will be collected pre-dose, and at 0.5, 1, 2, 2.5, 3, 4, and 5 hours after the start of infusion on Day 1. The actual sampling time will be recorded in the eCRF.

7.1.10 Systemic Haemodynamics

In Part 1 will systemic haemodynamis be assessed by measuring blood pressure and heart rate with non-invasive methods at screening, on Day -1, pre-dose and, 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours after start of infusion on Days 1-3, and in the morning of Day 4 as well as at the follow-up visit (see Section 7.1.2). In addition, PCWP, PAP, RAP, MAP and CO will be measured by a Swan-Ganz catheter introduced into the pulmonary artery pre-dose, and 2 hours after start of infusion on Day 3. CO, MAP, and RAP will be used for calculation of SVR.

In Part 2, blood pressure and heart rate will be measured with non-invasive methods at screening, on Day -1, pre-dose and, 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours after start of infusion on Day 1, and in the morning of Day 2 as well as at the follow-up visit (see Section 7.1.2). In addition, PCWP,

PAP, RAP, MAP and CO will be measured by a Swan-Ganz catheter introduced into the pulmonary artery pre-dose, and 2 hours after start of infusion on Day 1. CO, MAP, and RAP will be used for calculation of SVR.

7.1.11 Portal Haemodynamics

Measurements of portal haemodynamics to assess FHVP, WHVP, and IVC will be performed by hepatic vein catheterization pre-dose and at 1 and 2 hours after start of infusion on Day 3 in Part 1 and on Day 1 in Part 2. IVC will be measured pre-dose and at 2 hours after start of infusion only.

All measurements will be performed at least in duplicate (the average will be used for the endpoint), and permanent tracing will be obtained with a multichannel recorder and adequately calibrated transducers. The HVPG is calculated as the difference between the WHVP and FHVP.

Measurement of HVPG is a safe procedure and is used routinely in clinical practice in patients with chronic liver failure.

7.1.12 Body weight

Body weight for the assessment of any effect on possible ascites will be performed in Part 1 predose on Days 1-3 and in the morning on Day 4. In Part 2 it will be performed predose and in the morning on Day 2.

7.2 Other Assessments

7.2.1 Demographic and Baseline Data

Information about gender, date of birth, body weight, height, BMI, and medical history will be collected at the screening visit for each subject.

7.2.2 Physical Examinations

A physical examination will be performed at screening, to determine eligibility, and at the follow-up visit for both Part 1 and Part 2. The full physical examination comprises examination of general appearance, central and peripheral nervous system, head and neck (including ears, eyes, nose, mouth and throat), cardiovascular system, respiratory system, gastrointestinal system, lymphatic system, urinary system, skin and musculoskeletal system.

7.2.3 Serology

Determination of HIV-1 and HIV-2 antibodies, hepatitis B surface-antigen, and hepatitis C virus antibodies will be performed at screening for eligibility purposes.

7.2.4 Drug Screen and Alcohol Urine Test

To ensure that drugs are not abused, a urine dip-stick drug screen and an alcohol urine test will be performed at screening, and on Day -1 in both Part 1 and Part 2.

The drug screening will be performed from fresh mid-stream urine for the determination of amphetamines, benzodiazepines, cannabinoids, cocaine, ecstasy, opiates, and methadone.

7.2.5 Pregnancy Test

In women, serum β-hCG will be determined at screening, on Day -1 and at follow-up in both Part 1 and Part 2, using validated standard methods.

7.2.6 Prior and Concomitant Medication

Information about prior and concomitant medication will be collected for each subject throughout the study.

7.3 Drug Concentration Measurements

Analysis of plasma and urine concentrations of FE 204205, metabolite concentrations, and metabolite patterns will be performed under the responsibility of the Department of Bioanalysis at Ferring Pharmaceuticals A/S by means of a validated tandem mass spectrometry based (LC-MS/MS) method. The analysis will be performed within EU.

7.4 Handling of Biological Samples

A detailed description of all sample collections and shipment procedures will be included in a separate laboratory manual. The Sponsor or third party(ies) will store the blood samples up to 2 years after reporting of the trial.

8 ADVERSE EVENTS

8.1 Adverse Event Definition

An adverse event is any untoward medical occurrence in a subject participating in a clinical trial. It includes:

- Any unfavourable and unintended sign, symptom or disease temporally associated with the use of the IMP, whether or not considered to be caused by the IMP.
- Adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the IMP.
- Any laboratory abnormality, vital sign or finding from physical examination assessed as clinically significant by the Investigator [note: pre-existing conditions diagnosed through assessments and examinations at the screening visit or during the screening period are not adverse events, but are recorded as medical history.]
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.
- Overdoses and medication errors with and without clinical consequences.

Pre-treatment Adverse Event

A pre-treatment adverse event is any untoward medical occurrence arising or observed between signing of informed consent and the first administration of the IMP.

Treatment Emergent Adverse Event

A treatment emergent adverse event is any AE occurring after the administration of the IMP and within the time of residual drug effect, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after administration of the IMP and within the time of residual drug effect.

The time of residual drug effect is the estimated period of time after the administration of the IMP, where the effect of the product is still considered to be present based on pharmacokinetic (PK), pharmacodynamic (PD) or other substance characteristics. The residual drug effect is generally accepted to be 5 times the terminal half-life. The terminal half-life of FE 204205 is anticipated to within the range of 3 hours, i.e. in this study the residual drug effect is likely to be well within the time to the last assessment in both Part 1 and Part 2. Since this is the first time FE 204205 is administered to patients with cirrhotic portal hypertension, all AEs occurring from start of infusion on Day 1 up to the last assessment on Day 4 in Part 1 and Day 2 in Part 2 are regarded as treatment emergent.

Post-treatment Emergent Adverse Events

A post-treatment emergent adverse event is any AE occurring after the time of residual drug effect of the IMP, i.e. after the last assessment on Day 4 in Part1, and after Day 2 in Part 2.

8.2 Collection and Recording of Adverse Events

8.2.1 Collection of Adverse Events

The Investigator must monitor the condition of the subject throughout the trial from the time of obtaining informed consent until the last visit.

The sources of AEs cover:

- The subject's response to questions about his/her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit).
- Symptoms spontaneously reported by the subject.
- Investigations and examinations where the findings are assessed by the Investigator to be clinically significant changes or abnormalities.
- Other information relating to the subject's health becoming known to the Investigator (e.g. hospitalisation).

8.2.2 Recording of Adverse Events

The Investigator must record all AEs in the Adverse Event Log provided in each subject's eCRF with information about:

- AE
- Date and time of onset (time can be omitted, if applicable)
- Intensity
- Causal relationship to IMP
- Action taken to IMP
- Other action taken
- Date and time of outcome (time can be omitted, if applicable)
- Outcome
- Seriousness.

Each of the items in the Adverse Event Log is described in detail in the following sections.

Adverse Event

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a subject suffers from the same AE more than once and the subject recovers in between the events, the AEs should be recorded separately. If an AE changes in intensity, a worst-case approach should be used when recording the event, i.e. the highest intensity and the longest duration of the event. However, if an AE with onset before the first IMP administration (i.e. a pre-treatment adverse event) changes in intensity, this must be recorded as two separate events. The initial AE should be recorded with outcome "not yet recovered" and the date and time of outcome is when the intensity changed. The second AE should be recorded with date and time of onset when the intensity changed.

Note the following: A procedure is not an AE; the reason for conducting the procedure is. Hospitalisation is not an adverse event; the reason for hospitalisation is. Death is not an adverse event, but the cause of death is (an exception is sudden death of unknown cause, which is an adverse event).

Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the adverse event is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken or the examination was performed.

Intensity

The intensity of an adverse event must be classified using the following 3-point scale:

Mild: Awareness of signs or symptoms, but no disruption of usual activity.

Moderate: Event sufficient to affect usual activity (disturbing).

Severe: Inability to work or perform usual activities (unacceptable).

Causal Relationship to IMP

The possibility of whether the IMP caused the adverse event must be classified as one of the following:

Reasonable possibility:

There is evidence or argument to suggest a causal relationship between the IMP and the adverse event. The adverse event may occur as part of the pharmacological action of the IMP or may be unpredictable in its occurrence.

Examples:

- AEs that are uncommon but are known to be strongly associated with IMP exposure.
- AEs that are not commonly associated with IMP exposure, but the event occurs in association with other factors strongly suggesting causation, such as a strong temporal association or the event recurs on rechallenge.

No reasonable possibility:

There is no reasonable evidence or argument to suggest a causal relationship between the IMP and the adverse event.

Examples:

- known consequences of the underlying disease or condition under investigation.
- AEs common in the trial population, which are also anticipated to occur with some frequency during the course of the trial, regardless of IMP exposure.

Action Taken to IMP

The action taken to the IMP in response to an AE must be classified as one of the following:

- No change (medication schedule maintained or no action taken)
- Withdrawn
- Interrupted
- Dose reduced
- Dose increased.

Other Action Taken

AEs requiring therapy must be treated with recognised standards of medical care to protect the health and well-being of the subject. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the AE, this medication should be entered in the Concomitant Medication Log.

Date and Time of Outcome

The date and time the subject recovered or died.

Outcome

The outcome of an AE must be classified as one of the following:

- Recovered (fully recovered or the condition has returned to the level observed at initiation of trial treatment)
- Recovered with sequelae (resulted in persistent or significant disability/incapacity)
- Recovering (the event is improving)
- Not recovered
- Fatal.

8.3 Adverse Events of Special Interest

Taking into account the pharmacological profile of FE 204205, AEs related to exaggerated pharmacological effects, e.g. peripheral ischemia, increased blood pressure, and decreased cardiac output will be closely monitored.

8.4 Pregnancy and Pregnancy Outcome

If a pregnancy occurs, the subject will be withdrawn from the study and Global Pharmacovigilance at Ferring Pharmaceuticals informed, using an SAE Report Form. Note, that pregnancy itself is not an SAE. The mother and the foetus will be followed up at least until the birth of the infant and one month after the birth of the infant. In general, the follow-up will include the course, duration and the outcome of the pregnancy and the infant. If a pregnancy results in an abnormal outcome which the Investigator and/or Ferring consider to be related to the IMP, this outcome will be treated as an expedited report.

8.5 Serious Adverse Events

8.5.1 Serious Adverse Event Definition

Serious Adverse Events during the Trial

An event is defined a serious adverse event if it:	Guidance
results in **death**	Any event resulting in a fatal outcome must be fully documented and reported, including deaths occurring within four weeks after the treatment ends and irrespective of the causal relationship to the IMP. The death of a subject enrolled in a trial is *per se* not an event, but an outcome.
is **life-threatening**	The term life-threatening refers to an AE in which the subject was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death if it were more severe.
requires in-patient **hospitalisation** or prolongation of existing hospitalisation	The term hospitalisation means that the subject was admitted to hospital or that existing hospitalisation was extended as a result of an event. Hospitalisation describes a period of at least 24 hours. Over-night stay for observation, stay at emergency room or treatment on an out-patient basis do not constitute a hospitalisation. However, medical judgement must always be exercised and when in doubt the case should be considered serious (i.e. if case fulfils the criterion for a medically important event). Hospitalisations for administrative or social purposes do not constitute an SAE. Hospital admissions and/or surgical operations planned before trial inclusion are not considered adverse events, if the illness or disease existed before the subject was enrolled in the trial, provided that the condition did not deteriorate during the trial.
results in persistent or significant **disability/incapacity**	Disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions. In doubt, the decision should be left to medical judgement by the Investigator.

An event is defined a serious adverse event if it:	Guidance
is a **congenital anomaly/birth defect**	Congenital anomaly/birth defect observed in any offspring of the subject conceived during treatment with the IMP.
is an **important medical event**	Important medical events are events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of important medical events include AEs that suggest a significant hazard, contraindication or precaution, occurrence of malignancy or development of drug dependency or drug abuse. Medical and scientific judgement should be exercised in deciding whether events qualify as medically important. Important medical events include any suspected transmission of an infectious agent via a medicinal product. Any organism virus or infectious particle (e.g. prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a subject exposed to a medicinal product.

8.5.2 Collection, Recording and Reporting of Serious Adverse Events

SAE Reporting by the Investigator

All SAEs must be reported **immediately** to Ferring Global Pharmacovigilance as soon as it becomes known to the Investigator and not later than within 24 hours of their knowledge of the occurrence of an SAE.

The Investigator is responsible for submitting the completed SAE Report Form with the fullest possible details **within 3 calendar days** of his/her knowledge of the SAE.

The SAE Report Form is included in the eCRF system, and must be completed and submitted according to the instructions provided on the form. In case the eCRF cannot be accessed and hence the SAE Report Form cannot be filled in within the eCRF system, a paper SAE Report Form should be used and sent to Ferring Global Pharmacovigilance using the contact details below.

Global Pharmacovigilance, Ferring Pharmaceuticals A/S
E-mail: safety.mailbox@ferring.com
Fax: [redacted]

Completion of the Demographics, Adverse Event Log, Medical History Log and Concomitant Medication Log are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the eCRF for Ferring Global Pharmacovigilance to access the information.

Additional information relevant to the SAE such as hospital records, results from investigations, e.g. laboratory parameters (that are not already uploaded in the eCRF), invasive procedures, scans and x-rays, and autopsy results can be faxed or scanned and e-mailed to Ferring Global Pharmacovigilance using the contact details in the section above. In any case this information must be supplied by the Investigator upon request from Ferring. On any copies provided, such details such as subject's name, address, and hospital ID number should be concealed and instead subject number should be provided.

The Investigator will supply Ferring and the IEC with any additional requested information such as results of post-mortem examinations and hospital records.

Expedited Reporting by Ferring

Ferring will report all adverse events that are **serious, unexpected and with a reasonable possible causality to the IMP** as judged by either the Investigator or Ferring to the relevant parties within the stipulated timelines.

SAEs will be considered reportable regardless of whether or not the IMP was used in accordance with the provisions in the protocol, Investigator's Brochure and labelling.

Expedited Reporting

All AEs that are serious, unexpected and considered related to the IMP judged by either the Investigator or Ferring require expedited reporting. The expectedness is assessed by Ferring according to the Investigator's Brochure.

All available information relevant to the evaluation of the SAE will be reported. SAEs will be considered reportable regardless of whether or not the IMP was used in accordance with the provisions in the Protocol and/or the Investigator's Brochure. AEs that are serious but expected (related and/or unrelated to the IMP) are subject to expedited reporting in accordance with local requirements.

Timelines

Fatal or life-threatening serious, unexpected, related cases occurring in clinical investigations qualify for very rapid reporting. Regulatory agencies shall be notified (e.g., by telephone, facsimile transmission, or in writing) as soon as possible, but no later than 7 calendar days after first knowledge by the Sponsor that a case qualifies for expedited reporting, followed by a report that must be as complete as possible within 8 additional calendar days.

Serious cases that are unexpected and related and are not fatal or life-threatening, must be submitted as soon as possible but no later than 15 calendar days after first knowledge by the Sponsor that the case meets the minimum criteria for expedited reporting.

8.6 Follow-up of Adverse Events and Serious Adverse Events

8.6.1 Follow-up of Adverse Events with Onset during the Trial

During the trial, the Investigator must follow-up on each adverse event until it is resolved or until the medical condition of the subject is stable.

After the subject's last visit, the Investigator must follow-up on any AE classified as serious or considered to have a reasonable possible causality to the IMP until it is resolved or until the medical condition of the subject is stable. All such relevant follow-up information must be reported to Ferring. If the event is a chronic condition, the Investigator and Ferring may agree that further follow-up is not required.

8.6.2 Collection of Serious Adverse Events with Onset after Last Visit in the Trial

If an Investigator becomes aware of an SAE after the subject's last visit, and he/she assesses the SAE to have a reasonable possible causality to the IMP, the case will have to be reported to Ferring, regardless how long after the end of the trial this takes place.

9 STATISTICAL METHODS

The Global Biometrics Department of Ferring Pharmaceuticals A/S will be responsible for the statistical analyses. All analyses will be detailed in a separate Statistical Analysis Plan.

The pharmacokinetic parameters specified will be computed by the Department of Experimental Medicine, Ferring Pharmaceuticals A/S. Analysis of PK at end of study will be performed under the responsibility of Global Biometrics.

9.1 Determination of Sample Size

Part 1: No formal sample size calculation has been made. However, the number of patients (six) is considered sufficient to provide adequate information about the safety and tolerability of FE 204205, and thus inform about the suitable dose to evaluate in part 2.

Part 2: Since this is the first exploratory evaluation of the efficacy of FE 204205 on reducing portal pressure, the sample size and power calculations were performed by evaluation of the intra-group difference (before and after treatment with FE 204205). Assuming, based on the literature, a standard deviation (SD) of relative change from baseline of 12 % (see table below), a sample size of 14 patients on active treatment was deemed sufficient to detect a within-group 10 % reduction in HVPG, with $\alpha = 0.05$ and $1-\beta = 0.8$. Taking into consideration that it occasionally can be technically challenging to assess HVPG, a total of 20 patients (16 on FE 204205 and 4 on placebo) are targeted (4:1 randomisation).

Table 9-1 SD of relative (%) and absolute change from baseline in HVPG

Reference	Inv Drug	SDs of %Change in HVPG	SDs of Change in HVPG (mmHg)
Escorsell et al. (7)	Terlipressin	9%, 12%	
Abraldes et al. (8)	Simvastatin	9%, 12%	
Berzigotti et al. (9)	NCX-1000	(~13 %)	2.2 mmHg* (baseline 17.1 mmHg)
Reverter et al. (10)	Sapropterin	(~14 %)	2.2 mmHg* (baseline 16.0 mmHg)

*derived from reported mean change, samples size and p-value (two-sided paired t)

A reasonable estimate of the SD would be 12% (also assumed by Reverter et al. (10) in their power calculations)

9.2 Subject Disposition

All subjects screened and dosed in Part 1 and all subjects screened and randomised in Part 2 will be accounted for. All post-dosing and post-randomisation discontinuations will be summarised by time of, and reason for, discontinuation. The number of subjects screened but not found eligible will be stated in the report but otherwise not accounted for.

9.3 Protocol Deviations

The rating of protocol deviations will be decided on the basis of a review of the data before unblinding of the data and the declaration of 'clean file' and database lock. Major protocol deviation criteria will be specified in the statistical analysis plan.

9.4 Analysis Sets

9.4.1 Full Analysis Set

The full analysis data set (FAS) comprises all subject that are dosed at least once and is analysed according to the actuel treatment received.

9.4.2 Per Protocol Analysis Set

The per protocol (PP) analysis set comprises all dosed patients with no major protocol deviations and with HVPG ≥12 mmHg at baseline. For subjects encountering a major protocol deviation after study treatment has been initiated, data after the point at which the first deviation occurs will be excluded from all evaluations. Final decisions for exclusion of subjects/subject data from the PP analysis set will be made when a clean database is available to review ahead of database lock. The PP analysis set will be used for the PK analysis with the exception that specific subjects may be excluded if certain PK parameters cannot be estimated with sufficient precision (criteria for which will be further detailed in the Statistical Analysis Plan)

9.4.3 Safety Analysis Set

The safety analysis set comprises all subjects that received at least one dose of IMP and is analysed according to the actual treatment received.

9.5 Trial Population

Presentations will be based on the FAS and the PP datasets, if they are identical only the FAS dataset will be presented.

9.5.1 Demographics and other Baseline Characteristics

Descriptive statistics of demographics and other baseline characteristics will be presented separately for Part 1 and 2. For Part 2 also separately by treatment group to assess the degree of similarity achieved.

9.5.2 Medical History, Concomitant Medication and Other Safety Evaluations

Medical history (recorded at the screening visit) will be coded using the latest version of the MedDRA. Prior and concomitant medication will be summarised by ATC classification 1st level (alphabetically), ATC classification 2nd level (in decreasing order of frequency).

Concomitant medication and relevant medical history will be presented by descriptive statistics, by part and treatment.

9.6 Endpoint Assessments

Endpoint assessment will be based on the FAS data set and the PP dataset.

9.6.1 General Considerations

All endpoints (see Section 9.6.2) will be analysed using descriptive statistics, In general, the data will be presented by dose level and time point in part 1 and by treatment group and time point in Part 2.

Inferential analyses on change and relative change from baseline in systemic and portal haemodynamic variables from Part 2 of the study will be conducted by using an Analyses of Covariance (ANCOVA) adjusting for the respective baseline value per treatment arm and time point. The focussing on mean change from baseline is based on the assumption the of a negligible placebo effect as reported in e.g. (7,8). Nevertheless, placebo-subtracted treatment effects will also be inferred (despite of limited power) using ANCOVA with treatment group as an additional factor.

9.6.2 Endpoints

The following endpoints, without differentiation between primary and secondary endpoints, will be analysed:

- Type, frequency and intensity of adverse events (AEs)
- Electrocardiogram (ECG) (intervals, rhythm, and morphology)
- Clinical chemistry, haematology and urinalysis
- Venous blood gases including lactate
- Systemic haemodynamics: Blood pressures and heart rate by non-invasive measurements, pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure (PAP), right atrial pressure (RAP), mean arterial pressure (MAP), and cardiac output (CO) by pulmonary artery catheterisation. CO, MAP, and RAP will be used for calculation of the systemic vascular resistance (SVR)
- Portal haemodynamics by hepatic vein catheterization: Free hepatic venous pressure (FHVP), wedged hepatic venous pressure (WHVP), and inferior vena cava pressure (IVC). WHVP and the FHVP will be used for calculation of the hepatic venous pressure gradient (HVPG)
- Pharmacokinetics: AUC, AUC_t, % Extrap AUC, C_{max}, t_{max}, CL, V_z, λ_z, $t_{½}$, MRT, Ae and CL_R
- Metabolites in plasma and urine
- Blood and urinary biomarkers: Renin, aldosterone, norepinephrine, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), urine creatinine, urine sodium, and urine osmolality.

9.6.2.1 Systemic and portal haemodynamics

Supine blood pressure, pulse, and body temperature (absolute values) will be summarised by treatment and time point. Shift tables will be presented for changes from baseline, and will be

summarised by treatment. Abnormal values will be flagged in a subject listing. PCWP, PAP, RAP, MAP, CO, and SVR will be summarised by treatment and time point.

9.6.2.2 Electrocardiogram

All ECG data (heart rate, PR interval, RR, QRS, QT/QTc) will be listed for each subject and summarised by treatment and time point. In addition, ECGs will be categorised as "normal", "abnormal, not clinically significant", or "abnormal, clinically significant" (as judged by the Investigator) and summarised.

9.6.2.3 Clinical laboratory variables

Clinical chemistry and haematology parameters will be summarised in terms of absolute values and changes from baseline to each scheduled post-baseline time point. Shift tables will be presented for changes in category (below, within, or above the normal range) from baseline, presented by treatment. Urinalysis parameters will be summarised by treatment and time point.

9.6.2.4 Adverse events

Adverse events (AE) will be coded according to the latest version of MedDRA. All data will be listed by subject. Only treatment emergent AEs will be presented in summary tables. Separate data listing will be provided for AEs that are defined as pre-treatment or post-treatment emergent.

Overview of treatment emergent adverse events

A treatment emergent AEs (TEAE) overview summary table will be presented including for each treatment; the number of subjects reporting an AE, the percentage of subjects with an AE, and the number of events reported, for the following categories:

- All AEs
- Severe AEs
- SAEs
- Adverse drug reactions
- AEs leading to withdrawal
- Deaths

Adverse drug reactions (ADRs) are defined as events considered being reasonably possibly related to the IMP as judged by the Investigator.

Incidence of treatment emergent adverse events

Treatment-emergent adverse events will be summarised by MedDRA system organ class (SOC) and preferred term (PT). The summary will display the total number of subjects reporting an AE, the percentage of subjects (%) with an AE, and the number of events reported. AEs will be presented by SOC sorted alphabetically and PT sorted in decreasing frequency of occurrence (based on total across treatments). Summary tables will be prepared for:

- All TEAEs
- TEAEs by causality
- TEAEs by intensity
- ADRs by SOC and PT
- ADRs by intensity

Missing values will be treated as missing except for causality, intensity, seriousness, and outcome of an AE, at which occurrence a "worst case" approach will be taken. Thus, if causality is missing the AE will be regarded as related to the IMP, if the intensity is missing the intensity of the AE will be regarded as severe, if seriousness is missing the AE will be regarded as an SAE, and if the outcome is missing and no date of outcome is present the outcome is regarded as "not yet recovered".

Separate listings will be provided for SAEs, deaths, and other significant AEs, if any.

9.6.2.5 Venous blood gases including lactate

Venous blood gases and lactate will be summarised by treatment and time point.

9.6.2.6 Urinary output

Urinary output will be summarised for the collection interval by treatment and time point.

9.6.2.7 Blood and urinary biomarkers

Urine creatinine, urine sodium, and urine osmolality) will be summarised by treatment and time point.

Blood biomarkers (renin, aldosterone, norepinephrine, ANP, and BNP will be summarised by treatment and time point.

9.6.2.8 Pharmacokinetics

The pharmacokinetic analysis will be based on the PP analysis set and performed by Department of Experimental Medicine, Ferring Pharmaceuticals A/S. The pharmacokinetic parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® (Pharsight Corporation, U.S.A.). Actual sampling time points relative to dosing (i.e. starting time of dose infusion) will be used for the NCA and on the individual plots of plasma concentration versus time. Scheduled time points will be used in the descriptive summaries and summary plots of the data. Plasma concentration values below lower limit of quantification (LLOQ) and missing values (e.g. no blood sample collected or no value obtained at analysis) will be excluded from the NCA. Values below LLOQ will be represented by 'LLOQ/2 in summary tables and plots of the data. No formal analysis of "outliers" is planned.

From the plasma and urine concentration-time data of FE 204205 the following parameters will be estimated, if possible: AUC, AUC_t, % Extrap AUC, C_{max}, t_{max}, CL, V_z, λ_z, $t_{½}$, MRT, Ae and CL_R.

Selection of data points for calculation of $t_{½}$ via λ_z will be based on the following considerations:

- AUC will be calculated by the linear trapezoidal method
- The automatic range selection used in Phoenix WinNonlin® will be used to propose an optimal number of time points to use for the calculation of λ_z.
- At least three samples above LLOQ obtained during the log-linear elimination phase will be included in the calculation of the λ_z.
- The final selection of samples for calculation of λ_z will be based on visual inspection of log concentration-time plots of individual profiles.

Pharmacokinetic parameters will be presented by treatment (only for subjects receiving active treatment) with number of measurements, number of missing data, mean, standard deviation, median, minimum, maximum, geometric mean, and %CV (based on untransformed data) for geometric mean (for AUC and C_{max}). For $t_{½}$, the harmonic mean will be listed instead of the geometric mean. For t_{max} the geometric mean and the CV% will be omitted.

9.6.3 Other assessments

9.6.3.1 Physical examinations

Physical examination at each scheduled visit will be listed by subject.

9.7 Extent of Exposure and Treatment Compliance

Treatment compliance in terms of numbers of subjects that were dosed within each treatment regimen and the dose received will be presented for the PP and safety analysis sets.

9.8 Interim Analyses

No interim analysis is planned.

10 DATA HANDLING

10.1 Source Data and Source Documents

Source Data – ICH Definition

Source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents - ICH Definition

Source documents are defined as original documents, data, and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

Trial-specific Source Data Requirements – Ferring

Any trial specific source data will be described and defined in a Source Data Agreement form produced for this trial.

10.2 Electronic Case Report Form (eCRF)

An eCRF system provided by Target Health Inc. will be used for data capture. The system is fully validated and access at all levels to the system is granted/revoked following Ferring and vendor procedures, in accordance with regulatory and system requirements.

The eCRF system and the database will be hosted at Target Health Inc. After the trial database is declared clean and released to the statistician, a final copy of the database will be stored at Ferring within the SAS Drug Development system. The Investigator will also receive a copy of the trial site's final and locked data (including audit trail, electronic signature and queries) as write-protected pdf-files produced by Target Health Inc. The pdf-files will be stored on a CD and will be provided to the Investigator before access to the eCRF is revoked. The Investigator will approve/authorise the eCRF entries for each subject with an electronic signature which equals a handwritten signature. The signer must log in with his or her username and password and then re-enter this password on the page(s) requiring a signature. The data will be entered into the sytem in a timely manner.

Entry errors occurring in the eCRF will be corrected electronically. Such corrections/modifications will be automatically tracked by an audit trail detailing the date and time of the correction and the name of the person making the correction.

10.3 Data Management

All data management activities will be specified in a Data Management Plan prepared under the responsibility of the Global Biometrics Department, Ferring Pharmaceuticals A/S. The data management plan will be issued before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning and validation. Alla data management will be performed by Target Health Inc. under the responsibility of the Global Biometrics Department, Ferring Pharmaceuticals A/S. A study database will be created according to the data management standard operating procedures and data validation programmes will be developed to check for data completion and validity.

Laboratory datawill be transferred electronically to Target Health Inc. for inclusion in the study database according to laboratory data transfer specifications to be agreed between the individual laboratories and Target Health Inc. For medical coding of AEs, medical history, and concomitant medication the most recent versions of MedDRA and WHO Drug will be used. The coding will be performed by Ferring Pharmaceuticals A/S. When all data have been processed, queried resolved, medical coding completed and any issues from review of protocol deviations and data listings resolved, the database will be locked and any further update will be denied. A final quality assurance audit of the locked database will take place prior to transfer of the final database structure according to Ferring's data transfer specifications.

10.4 Provision of Additional Information

On request, the Investigator will provide Ferring with additional data relating to the trial, or copies of relevant source records, duly anonymised and protected in accordance with applicable requirements.

11 MONITORING PROCEDURES

11.1 Periodic Monitoring

The Monitor will contact and visit the Investigator periodically to ensure adherence to the protocol, International Conference of Harmonisation-Good Clinical Practice (ICH-GCP), standard operating procedures and applicable regulatory requirements, maintenance of trial-related source records, completeness, accuracy and verifiability of eCRF entries compared to source data, verification of drug accountability and compliance to safety reporting instructions. The Investigator will permit the Monitor direct access to all source data, including electronic medical records, and/or documents in order to facilitate data verification. The Investigator will co-operate with the Monitor to ensure that any discrepancies that may be identified are resolved. The Investigator is expected to be able to meet the Monitor during these visits. When the first subject is randomised at the trial site a monitoring visit will take place shortly afterwards.

11.2 Audit and Inspection

The Investigator will make all the trial-related source data and records available at any time to quality assurance auditor(s) mandated by Ferring, or to domestic/foreign regulatory inspectors or representatives from IECs who may audit/inspect the trial.

The main purposes of an audit or inspection are to assess compliance with the trial protocol and the principles of ICH-GCP including the Declaration of Helsinki and all other relevant regulations.

The subjects must be informed by the Investigator and in the Informed Consent Documents that authorised Ferring representatives and representatives from Regulatory Authorities and IECs may wish to inspect their medical records. During audits/inspections the auditors/inspectors may copy relevant parts of the medical records. No personal identification apart from the screening/randomisation number will appear on these copies.

The Investigator should notify Ferring without any delay of any inspection by a Regulatory Authority or IEC.

11.3 Confidentiality of Subject Data

The Investigator will ensure that the confidentiality of the subjects' data will be preserved. In the eCRF or any other documents submitted to Ferring, the subjects will not be identified by their names, but by an identification system, which consists of an assigned number in the trial. Documents that are not for submission to Ferring, e.g. the confidential subject identification code and the signed Informed Consent Documents, will be maintained by the Investigator in strict confidence.

12 CHANGES IN THE CONDUCT OF THE TRIAL

12.1 Protocol Amendments

Any change to this protocol will be documented in a protocol amendment, issued by Ferring, and agreed upon by the Investigator and Ferring prior to its implementation. Amendments may be submitted for consideration to the approving IEC and Regulatory Authorities, in accordance with local regulations. Changes to the protocol to eliminate immediate hazard(s) to trial subjects may be implemented prior to IEC approval/favourable opinion.

12.2 Deviations from the Protocol

If deviations from the protocol occur, the Investigator must inform the Monitor, and the implications of the deviation must be reviewed and discussed. Any deviation must be documented, either as answer to a query in the eCRF, in a protocol deviation report or a combination of both. A log of protocol deviation reports will be maintained by Ferring. Protocol deviation reports and supporting documentation must be kept in the Investigator's File and the Trial Master File.

12.3 Premature Trial Termination

Both the Investigator (with regard to his/her participation) and Ferring reserve the right to terminate the trial at any time. Should this become necessary, the procedures will be agreed upon after consultation between the two parties. In terminating the trial, Ferring and the Investigator will ensure that adequate consideration is given to the protection of the best interests of the subjects. Regulatory Authorities and IEC will be informed.

13 REPORTING AND PUBLICATION

13.1 Clinical Trial Report

The data and information collected during this trial will be reported in a clinical trial report prepared by Ferring and submitted for comments and signature to the signatory Investigator.

13.2 Confidentiality and Ownership of Trial Data

Any confidential information relating to the IMP or the trial, including any data and results from the trial will be the exclusive property of Ferring. The Investigator and any other persons involved in the trial will protect the confidentiality of this proprietary information belonging to Ferring.

13.3 Publications and Public Disclosure

13.3.1 Publication Policy

At the end of the trial, one or more manuscripts for joint publication may be prepared in collaboration between the Investigator(s) offered authorship and Ferring.

Authorship is granted based on the ICMJE criteria (see current official version: http/www.ICMJE.org). The total number of authors is based on the guideline from the relevant journal or congress. In the event of any disagreement in the content of a publication, both the Investigator's and Ferring's opinion will be fairly and sufficiently represented in the publication.

Any external Contract Research Organisation or laboratory involved in the conduct of this trial has no publication rights regarding this trial.

13.3.2 Public Disclosure Policy

It is the responsibility of Ferring to register the trial in appropriate public registry/registries according to applicable regulations.

14 ETHICAL AND REGULATORY ASPECTS

14.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

An IEC will review the protocol and any amendments and advertisements used for recruitment. The IEC will review the Subject Information Sheet and the Informed Consent form, their updates (if any), and any written materials given to the subjects. A list of all IECs to which the protocol has been submitted and the name of the committee chairmen will be included in the Clinical Trial Report.

14.2 Regulatory Authority(ies) Authorisation / Approval / Notification

The regulatory permission to perform the trial will be obtained in accordance with applicable regulatory requirements. All ethical and regulatory approvals must be available before a subject is exposed to any trial-related procedure, including screening tests for eligibility.

14.3 End-of-Trial and End-of-Trial Notification

At the end of trial, the sponsor will notify the appropriate Regulatory Authority and IEC about the study completion. End of trial is defined as last subject last visit. In addition, a clinical summary report will be provided within one year of trial completion.

14.4 Ethical Conduct of the Trial

This trial will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, ICH-GCP and applicable regulatory requirements.

14.5 Subject Information and Consent

The Investigator (or the person delegated by the Investigator) will obtain a freely given written consent from each subject after an appropriate explanation of the aims, methods, anticipated benefits, potential risks, and any other aspects of the trial which are relevant to the subject's decision to participate. The trial subject must be given ample time to consider participation in the trial, before the consent is obtained. The Informed Consent Documents must be signed and dated by the subject and the Investigator who has provided information to the subject regarding the trial before the subject is exposed to any trial-related procedure, including screening tests for eligibility.

The Investigator (or the person delegated by the Investigator) will explain that the subject is completely free to refuse to enter the trial or to withdraw from it at any time, without any consequences for his/her further care and without the need to justify his/her decision.

The subject will receive a copy of the Subject Information and his/her signed Informed Consent form.

If new information becomes available that may be relevant to the trial subject's willingness to continue participation in the trial, a new Subject Information and Informed Consent form will be

forwarded to the IEC (and Regulatory Authorities, if required). The trial subjects will be informed about this new information and re-consent will be obtained.

14.6 Subject Participation Card

The subject will be provided with a Subject Participation Card bearing the following information:

- That he/she is participating in a clinical trial.
- That he/she is treated with FE 2014205 (note: in case of a blinded trial, treatment allocation must not be revealed on the Subject Participation Card).
- The name and phone number of the Investigator.
- The name, address and phone number of Ferring (as required by local regulations).

The subject will be asked to keep the Subject Participation Card in their possession at all times during the trial and to return it at the last trial visit, if applicable.

Additionally, each subject's primary care physician will be notified of their participation in the trial by the Investigator, if the subject agrees and if applicable.

14.7 Compliance Reference Documents

The Declaration of Helsinki, the consolidated ICH-GCP, the EU Clinical Trials Directive and other national law(s) in the country where the trial takes place shall constitute the main reference guidelines for ethical and regulatory conduct.

15 LIABILITIES AND INSURANCE

15.1 ICH-GCP Responsibilities

The responsibilities of Ferring, the Monitor and the Investigator are defined in the ICH-GCP consolidated guideline, and applicable regulatory requirements in the country where the trial takes place. The Investigator is responsible for adhering to the ICH-GCP responsibilities of Investigators, for dispensing the IMP in accordance with the approved protocol or an approved amendment, and for its secure storage and safe handling throughout the trial.

15.2 Liabilities and Insurance

In case of any damage or injury occurring to a subject in association with the IMP or the participation in the trial, Ferring has contracted an insurance which covers the liability of Ferring, the Investigator and other persons involved in the trial in accordance with applicable laws and regulations.

16 ARCHIVING

16.1 Investigator File

The Investigator is responsible for maintaining all the records, which enable the conduct of the trial at the site to be fully understood, in compliance with ICH-GCP. The trial documentation including all the relevant correspondence should be kept by the Investigator for at least 25 years after the completion or discontinuation of the trial, if no further instructions are given by Ferring.

The Investigator is responsible for the completion and maintenance of the confidential subject identification code which provides the sole link between named subject source records and anonymous eCRF data for Ferring. The Investigator must arrange for the retention of this Subject Identification Log and signed Informed Consent Documents for at least 25 years after the completion or discontinuation of the trial.

No trial site document may be destroyed without prior written agreement between the Investigator and Ferring. Should the Investigator elect to assign the trial documents to another party, or move them to another location, Ferring must be notified. If the investigator retires and the documents can no longer be archived by the site, Ferring can arrange having the Investigator File archived at an external archive.

16.2 Trial Master File

Ferring will archive the Trial Master File in accordance with ICH-GCP and applicable regulatory requirements.

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