Academic and Clinical Central Office for Research and Development

CLINICAL STUDY REPORT

Serelaxin To Lower Portal Pressure in Patients with Cirrhosis and Portal Hypertension (STOPP)

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INDEX PAGE

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STUDY START DATE: 19th Oct 2017

STUDY END DATE: 31st Aug 2018

IMP: Serelaxin (recombinant human relaxin-2)

1a. Title: A Phase 2 Randomised Controlled Trial of Serelaxin to Lower Portal Pressure in Cirrhosis (STOPP)

1b. Abstract

Background: Portal hypertension underlies most of the serious complications of liver cirrhosis but pharmacological treatment options are limited. Therapeutic reduction of portal hypertension improves clinical outcomes, including risk of hepatic decompensation and death. Data collected from preclinical models and a small exploratory open-label phase 2 clinical study in patients with cirrhosis showed that serelaxin reduced portal pressure.
Methods: In a randomised, double-blind, placebo-controlled phase 2 study conducted in a single centre (Royal Infirmary of Edinburgh, Edinburgh, UK), male and female adult patients with cirrhosis and clinically-significant portal hypertension (hepatic venous pressure gradient (HVPG) >10 mmHg) were enrolled to study the effects of serelaxin on portal and systemic haemodynamics. Participants were allocated to serelaxin or placebo in a 3:1 ratio. The primary endpoint was the change from baseline in fasting HVPG after 2 hr peripheral intravenous serelaxin infusion (80 µg/kg/day for 60 minutes followed by 30 µg/kg/day for at least 60 minutes). Secondary endpoints included the change from baseline in hepatic blood flow (measured by indocyanine green clearance) and systemic haemodynamics (cardiac index and systemic vascular resistance index by impedance cardiography; aortic pulse wave velocity). Short-term safety and tolerability of serelaxin infusion was also assessed. There was no longterm follow-up.
Results: A total of 17 participants were screened. Of these, 2 had a screening failure and did not proceed to randomisation. Fifteen patients were randomised and 11 completed the trial (n=9 serelaxin, n=2 placebo). Reasons for withdrawal were baseline HVPG <10 mmHg (n=2) and technical failure (n=2). Median age was 56 (range 43-69) and 73% were male. Cirrhosis aetiologies were alcohol (n=10), non-alcoholic fatty liver disease (n=2), hepatitis C (n=2) and hepatitis B (n=1). Subjects were Child-Pugh class A (60%) and B (40%) with median MELD score of 10 (range 6-14). Mean baseline HVPG was 16.3 mmHg (range 10.3-21.7). There was no significant change from baseline in HVPG after 2 hr serelaxin infusion (mean±SD 0.4±3.5 mmHg; p=0.76). There were also no significant changes from baseline in hepatic blood flow or systemic haemodynamic assessments following serelaxin. Treatment with serelaxin was well-tolerated. Overall, 12 adverse events were reported in 7 subjects treated with serelaxin. Most were non-serious and considered unrelated to the IMP. There were no serious adverse events.
Conclusions: In a small exploratory randomised study in patients with cirrhosis and clinically-significant portal hypertension, serelaxin infusion was safe and well-tolerated but had a neutral effect on portal pressure. Future studies could evaluate effects in patients with less severe portal hypertension (HVPG 5-10 mmHg), in whom intrahepatic vascular resistance mainly contributes to increased portal pressure.

2. Introduction

2a. Background

Standardized mortality rates for liver disease in the UK have increased 400% since 1970, and in people younger than 65 years have increased by almost 500% (Williams R et al, Lancet 2014). In patients with cirrhosis of the liver, portal hypertension is the main cause of death and of liver transplantation. In Europe alone it is estimated that 29 million patients suffer from chronic liver disease, and that 170,000 die each year from complications of cirrhosis, a number exceeding the mortality due to breast cancer (Blachier M et al, J Hepatol 2013). Patients with a hepatic venous pressure gradient (HVPG) >10 mmHg are at increased risk of hepatic decompensation (Garcia-Tsao G et al, Hepatology 1985) and of hepatocellular carcinoma (Ripoll C et al, J Hepatol 2009). Variceal bleeding occurs when the HVPG is >12 mmHg. A reduction in HVPG to <12 mmHg or by >20% from baseline are reported to improve clinical outcomes and represent targets for haemodynamic response in interventional studies (Garcia-Tsao G & Bosch J, NEJM 2010). Despite a significant improvement in outcomes over the past 30 years, the average 6-week mortality of the first episode of variceal bleeding in most studies is reported to be up to 20% (Tripathi D et al, Gut 2015).

Terlipressin, a synthetic analogue of vasopressin, has an immediate systemic vasoconstrictor action followed by portal haemodynamic effects due to slow conversion to vasopressin. It is the only pharmacological agent used in acute variceal bleeding that has been shown to reduce mortality in placebo-controlled trials (Tripathi D et al, Gut 2015). Terlipressin decreases failure of initial haemostasis by 34%, decreases mortality by 34%, and is considered a first-line treatment for bleeding oesophageal varices, when available. However, off-target effects include peripheral and coronary ischaemia, and adverse events (AEs) occur in 10-20% of patients (Krag A et al, Adv Ther 2008). Terlipressin is not licensed in the USA, where octreotide (a somatostatin analogue) is most commonly used. Octreotide is also thought to act as a mesenteric arterial vasoconstrictor, but in an acute haemodynamic study, octreotide was found to only transiently reduce HVPG and portal venous flow (Baik S et al, Am J Gastroenterol 2005). Nevertheless, octreotide has recently been shown to be as effective as terlipressin in the control of acute variceal bleeding (Seo Y et al, Hepatology 2014).

We had previously shown that serelaxin, a recombinant form of the human peptide hormone relaxin-2, elicited anti-fibrotic and portal hypotensive effects in cirrhotic rats (Fallowfield JA et al, Hepatology 2014). Moreover, serelaxin reduced portal pressure by decreasing intrahepatic vascular resistance through augmentation of nitric oxide (NO) bioavailability and signaling, thus maintaining or enhancing hepatic blood flow. In a small exploratory open-label phase 2 study (EudraCT no. 201200023626, REC ref 12/SS/0177), Part B showed that serelaxin induced a rapid and potentially clinically significant reduction in portal pressure in patients with cirrhosis, portal hypertension and a TIPSS. Following at least 120 minutes of serelaxin infusion there was a 31.3% (95% CI -66.5, 71.6) reduction in the portal pressure gradient (PPG) compared to baseline. During the infusion there was a progressive reduction in the portal vein pressure (PVP) reaching a decrease of 25.2% (95% CI -12.7, 50.3) from baseline at the 120-minute time point. The reduction in PVP started at 30 minutes and continued through to the 135-minute time point. With serelaxin infusion, there were no newly occurring liver enzyme abnormalities, no clinically significant changes in blood pressure, and no discontinuations due to AEs. Indeed, in a separate study the pharmacokinetic and safety profiles of serelaxin were not affected in patients with mild, moderate or severe hepatic impairment (Kobalava Z et al, Br J Clin Pharmacol 2014).

Variceal bleeding and bacterial infections (that frequently occur in patients with cirrhosis and upper gastrointestinal haemorrhage) can precipitate type-1 hepatorenal syndrome, which carries a very high mortality rate. Emerging data suggested that serelaxin might also have renoprotective properties. The

beneficial renal haemodynamic effects of serelaxin (increased renal blood flow (RBF) and reduced filtration fraction) were shown in patients with chronic heart failure (Voors A et al, Circ Heart Fail 2014) and improvement of renal biomarkers (creatinine and cystatin-C) in patients with acute heart failure (Metra M et al, J Am Coll Cardiol 2013). Furthermore, in a Novartis-sponsored phase 2 study (EudraCT no. 201200023626, REC ref 12/SS/0177), Part A showed using 3-D phase contrast magnetic resonance angiography that 120 minutes of serelaxin infusion increased RBF by 65.4% from baseline in patients with cirrhosis and portal hypertension. Serelaxin decreased blood flow in the portal vein (PV) by 11.9%, increased blood flow in the hepatic artery by 18%, but had no effect on superior mesenteric artery (SMA) flow. In contrast, terlipressin markedly reduced SMA flow (36.9%), PV flow (40%) and total liver blood flow (34.7%). Importantly, there was no clinically significant decrease in blood pressure with serelaxin and no difference between pre and post treatment peripheral plasma NO levels.
The potential therapeutic profile of serelaxin (reduction in portal pressure, preserved or increased hepatic blood flow, renal vasodilation, anti-fibrotic) indicates that it may have important effects in patients with chronic liver disease. This randomised placebo-controlled pilot study was conducted to evaluate the effect of serelaxin on HVPG and hepatic blood flow in patients with cirrhosis and portal hypertension.

2b. Objectives

The aim of this exploratory study was to investigate the safety and efficacy of serelaxin in reducing portal pressure, as determined by the hepatic venous pressure gradient (HVPG), in patients with compensated cirrhosis and portal hypertension.

Primary Objective

- To demonstrate that serelaxin induces a clinically significant acute reduction in portal pressure of at least 20% from baseline in patients with cirrhosis and portal hypertension

Secondary Objectives

- To determine the effect of serelaxin on hepatic blood flow
- To determine the effect of serelaxin on systemic haemodynamics
- To collect safety and tolerability data for serelaxin

3. Methods

3a. Trial design

This study is a single site, phase 2, double-blind, randomised, parallel group trial to investigate the effects of serelaxin on portal hypertension in patients with liver cirrhosis. Participants were allocated to receive serelaxin or placebo in a 3:1 ratio using block randomisation with a random block size; there was no stratification to this allocation. The control group was used to maintain the blind and provide information to aid the design of future studies and no statistical comparison between treatment and control groups was planned.

3b. Changes to trial design

Not applicable.

4. Participants

INCLUSION CRITERIA:

1) **Male or female adult subjects** over 18 years of age
2) Able to provide written informed consent and able to understand and willing to comply with the requirements of the study
3) Clinical/imaging-diagnosed or biopsy-proven **liver cirrhosis** of any aetiology
4) Evidence of **portal hypertension** either on imaging or previous endoscopy
5) Patients with large/grade 3 varices as identified by endoscopy within 6 months of screening must be in an endoscopic band ligation programme at the time of study entry
6) Suspected **hepatic venous pressure gradient (HVPG) ≥10 mmHg** at baseline

EXCLUSION CRITERIA:

1) Pregnancy or nursing (lactating) women
2) Women of child-bearing potential not using highly effective methods of contraception.
3) Severe liver failure defined by one of the following: Prothrombin activity < 40%, Bilirubin > 5 mg/dL (85 μmol/L), hepatic encephalopathy > grade I
4) Presence of any non-controlled and clinically significant disease that could affect the study outcome or that would place the patient at undue risk
5) A history of variceal bleed within 1 month prior to visit 1
6) Hepatocellular carcinoma or history of malignancy of any organ system (other than localized basal cell carcinoma of the skin) treated or untreated.
7) Portal vein thrombosis
8) Previous surgical shunt or TIPSS
9) Current use of beta-blockers or nitrates, any other drug therapy known to have an influence on portal pressure (diuretics permitted provided patients have been on a stable dose for at least 30 days)
10) History of drug or alcohol abuse within 1 month of enrolment
11) Sitting Systolic Blood Pressure <110 mmHg at screening visit or within 10 minutes prior to starting study drug infusion.
12) Use of other investigational drugs within 5 half-lives of enrolment, or within 30 days/until the expected pharmacodynamic effect has returned to baseline, whichever is longer
13) Significant arrhythmias, which include any of the following: sustained ventricular tachycardia, bradycardia with sustained ventricular rate < 45 beats per minute or atrial fibrillation/flutter with sustained ventricular response of > 90 beats per minute at rest, or Long QT syndrome or QTc > 450 msec (QT correction will be performed using the Fredericia correction method: QTcF = QT/RR0.33) for males and > 460 msec for females at screening (visit 1).
14) Documented hypersensitivity to intravenous contrast agents and/or iodine
15) Severe renal impairment (eGFR<30 mL/min /1.73 m^2)
16) Significant left ventricular outflow tract obstructions (e.g., severe valvular aortic stenosis, obstructive cardiomyopathy), severe mitral stenosis, restrictive amyloid myocardiopathy, acute myocarditis
17) Severe aortic or mitral regurgitation for which surgical or percutaneous intervention is indicated
18) Major neurologic event including cerebrovascular events, within 30 days prior to screening
19) Clinical evidence of acute coronary syndrome currently or within 30 days prior to enrolment
20) History of hypersensitivity to study drug serelaxin or study drug ingredients
21) Inability to follow instructions or comply with follow-up procedures
22) Pacemaker, cardiac resynchronisation device or implantable cardioverter-defibrillator *in situ*

5. Study setting

This was a single site study, undertaken at the Royal Infirmary of Edinburgh, Edinburgh, UK.

Participants attended the Royal Infirmary of Edinburgh Clinical Research Facility (RIE-CRF) for screening (**visit 1**) for less than 60 min consisting of physical examination, screening blood tests (full blood count, coagulation and biochemistry), ECG, blood pressure measurement, and informed consent.

Randomisation was performed once it was known that the participant had passed screening, prior to the study visit.

At the study visit (**visit 2**; ≤7 days after the screening visit), eligible participants attended the RIE-CRF and had baseline haemodynamic measurements performed, following an overnight fast and the avoidance of caffeine for >8 hr. After baseline evaluation and confirmation of HVPG ≥10 mmHg, participants received (in a double-blind fashion) either serelaxin or placebo. The haemodynamic measurements were repeated at specified timepoints (Fig 1). A peripheral blood sample was taken at baseline and after 2h, processed, and stored for future analysis. After the post-treatment assessments, participants were observed for a recovery period of 4h which included physical examination, blood pressure, ECG measurement and routine laboratory blood tests.

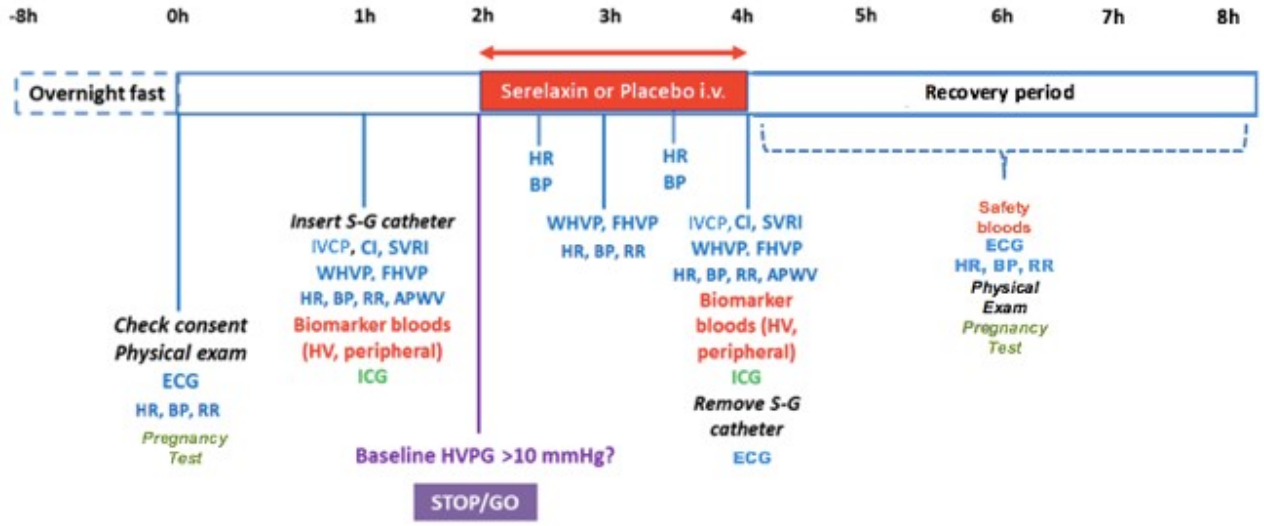


Fig 1. Study visit overview.

ECG, electrocardiogram; HR, heart rate; BP, blood pressure; RR, respiratory rate; S-G, Swan-Ganz; IVCP, inferior vena cava pressure; CI, cardiac index; SVRI, systemic vascular resistance index; APWV, aortic pulse wave velocity; WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; ICG, indocyanine green; HV, hepatic vein

6. Interventions

IMP: Serelaxin (recombinant human relaxin-2).

Placebo: The placebo used is 20 mM sodium acetate buffer solution at pH 5.0 with an appearance identical to serelaxin to achieve blinding.

TABLE 1: Summary of Planned Investigational and Reference Therapy

Treatment Arm	# of Patients Entered Treatment	Type of Study Drug	Compound	Min Dose	Max Dose	Frequency	Admin. Route	Generic Acceptable? (applies only for comparator)
Arm 1	**15**	Investigational	Serelaxin (RLX030)	30 µg/kg/d	80 µg/kg/d	Continuous infusion 2h	intravenous	-
Arm 2	**5**	Comparator	Placebo	-	-	Continuous infusion 2h	intravenous	No

7. Outcomes

7a. Primary Endpoint

- Change from baseline in fasting hepatic venous pressure gradient (HVPG) after 2 hr serelaxin infusion

HVPG measurement: Portal pressure was measured indirectly by determining the HVPG as previously described (ref). The procedure was performed after fasting all night and at roughly the same time of day due to circadian variation in HVPG measurements. Using the femoral approach, a balloon-tipped catheter was advanced into a hepatic vein under fluoroscopic guidance. The free hepatic venous pressure (FHVP) was measured with the balloon deflated and floating freely in the hepatic vein close to its junction with the inferior vena cava. The wedged hepatic venous pressure (WHVP) was measured with the balloon inflated until the branch of hepatic vein was completely occluded. HVPG was obtained by subtracting the FHVP from the WHVP. All measurements were performed in triplicate and a permanent record of the tracings were obtained.

7b. Secondary Endpoints

- Change from baseline in fasting HVPG after 1 hr serelaxin infusion
- Change from baseline in fasting hepatic blood flow after 2 hr serelaxin infusion (measured from the concentration of indocyanine green (ICG) in the hepatic venous blood vs peripheral venous blood using the Fick Principle)
- Change from baseline in inferior vena cava pressure (IVCP) after 2 hr serelaxin infusion
- Change from baseline in cardiac index (CI) after 2 hr serelaxin infusion
- Change from baseline in systemic vascular resistance index (SVRI) after 2 hr serelaxin infusion
- Change from baseline in aortic pulse wave velocity after 2 hr serelaxin infusion
- Safety and tolerability of serelaxin infusion (as assessed throughout the study by monitoring AEs, clinical laboratory blood tests, heart rate, blood pressure and ECG)
- Change from baseline in blood biomarker measurements after 2 hr serelaxin infusion

7c. Changes to outcomes

It was decided only to measure mechanistic blood biomarkers if the primary endpoint data was positive. In the event of a neutral study result, the blood samples would be banked and stored for ethically-approved research in the future.

8. Sample size

The primary efficacy endpoint was the decrease in fasting HVPG between baseline and 2h post serelaxin treatment, targeting for a 20% reduction. The sample size calculation was based on a previous study in Edinburgh evaluating carvedilol (Tripathi D et al, Aliment Pharmacol Ther 2002) and the data from the previous Novartis-sponsored serelaxin phase II study (RLX030X2201). Assuming a mean baseline HVPG of 16.37 (SD=2.14) mmHg and post-baseline HVPG of 13.1 (SD=3.91) mmHg (20% decrease), the change from baseline in HVPG was estimated to be 3.3 (SD=4) mmHg. A sample size of 14 subjects in the serelaxin group provides 80% power to detect at least a 20% decrease from baseline in HVPG using a two-sided paired t-test with alpha level 0.05. A small number of placebo-treated patients were included in order to preserve double-blindness, not as a comparison group. Therefore, it was proposed that a total of 20 patients (15 serelaxin and 5 placebo) would be randomised in a 3:1 ratio.

9. Interim analyses and stopping guidelines

There was no planned formal interim analysis and there was no Data Monitoring Committee for this study.

10. Randomisation

10a. Randomisation Procedures

Participants were randomised to receive either serelaxin or placebo. Both treatments were prepared to be similar in appearance, colour, and organoleptic properties. Masking was double-blind.

Randomisation was carried out after it was confirmed that the participant had passed screening, prior to the study visit (visit 2). The randomisation service was carried out by the Edinburgh Clinical Trials Unit (ECTU), allowing researchers and participants to remain blinded to treatment allocation. Blocked randomisation was used to achieve balance between study arms and to reduce the opportunity for bias and confounding. Random sequences of block sizes were generated by computer to achieve a 3:1 allocation ratio between serelaxin and placebo (i.e. n=15:5). Pharmacy prepared the appropriate treatment after randomisation.

Serelaxin and placebo were administered, in a double-blind manner, via i.v. infusion at two different infusion rates: 80 µg/kg/day for 60 minutes followed by 30 µg/kg/day for at least 60 minutes (until completion of the final HVPG/ICG measurements). This was achieved by a single infusion bag with a change in the administration rate.

10b. Withdrawal of Study Participants

Participants were withdrawn from the study if the baseline HVPG measurement was <10 mmHg (as per Fig 1). If upon randomisation it was established that the baseline HVPG was <10 mmHg, the participant would then meet the withdrawal criteria and the study visit was abandoned.
Participants were able to voluntarily withdraw from the study for any reason at any time. If premature withdrawal occurred for any reason, the investigator made every effort to determine the primary reason for a participant's premature withdrawal from the study and recorded this information on the CRF.
The investigator would also discontinue the study treatment for a given participant or withdraw the participant from study if, on balance, he/she believed that continuation would be detrimental to the participant's well-being.

Study treatment would therefore be discontinued under the following circumstances:

- Withdrawal of informed consent
- Emergence of clinically significant adverse events at the discretion of the investigator
- Any other protocol deviation that results in a significant risk to the patient's safety
- Signs or symptoms of hypotension, or blood pressure less than either SBP <90 and/or DBP <60 mmHg would be thoroughly evaluated by the investigator and the patient permanently discontinued from study drug
- If pregnancy was diagnosed at any point during the study.

Patients prematurely withdrawn from the study could be replaced by an equal number of newly enrolled patients only if they were discontinued prior to the completion of the final set of haemodynamic measurements (Fig 1).

11. Statistics

11a. Statistical methods

Summary statistics (n, mean, SD, median, min, max, Q1 and Q3) are presented over time for the baseline, post-baseline and change from baseline measurements for the primary endpoint in the serelaxin and placebo group. The geometric mean is presented for the baseline value, post-baseline values, and for the ratio to the baseline values. Confidence intervals are calculated for both the arithmetic and geometric means. Paired t-test is used to test the mean change from baseline measurements. The secondary endpoints (e.g. hepatic blood flow) are subjected to the same analysis as the HVPG.

The placebo control group was used to maintain the blind. We present the baseline to 2 hr change in the same way as the primary outcome although as this has not been powered for, no direct statistical comparison is made between serelaxin and placebo. The proposed analyses will help gain valuable information for designing a future larger randomised controlled trial.

11b. Overall Statistical Principles

All participants are analysed in the group to which they were originally assigned irrespective of the treatment received with the exception of adverse events which are presented according to allocated treatment and also treatment received. For all analysis unless otherwise specified statistical significance

is taken to be $p<0.05$. For all analysis where a paired t-test is stipulated this is performed for both mean (arithmetic) and also geometric means.

11c. Timing of Final Analysis

Statistical analysis was performed after all data entry had been performed, after any 'cleaning' that was required had been completed and the database locked.

11d. Missing Data

Any missing data as a consequence of the participant not having post-baseline measurement was not imputed and participants with missing post-baseline data are excluded from the analysis at that time point. The number of participants who withdrew during the course of the study is presented broken down by treatment allocation and presented with reasons for withdrawal where available.

11e. Protocol violations/deviations, compliance

Protocol violations/deviations as captured in the sponsors database are presented in a tabular format.

11f. Adverse Events

Safety assessments included collection of adverse events (AE), clinical examination, vital signs, laboratory tests and electrocardiograms (ECGs). Both the severity of AEs and relation to study medication treatment was collected.

The number and percent of participants experiencing AEs are presented. There were no serious adverse events (SAEs) reported. A line listing for all AEs is presented broken down by treatment allocation and also presented broken down by treatment received. The number of AEs per participant is presented descriptively and in appropriate cross tabulations with severity and relatedness.

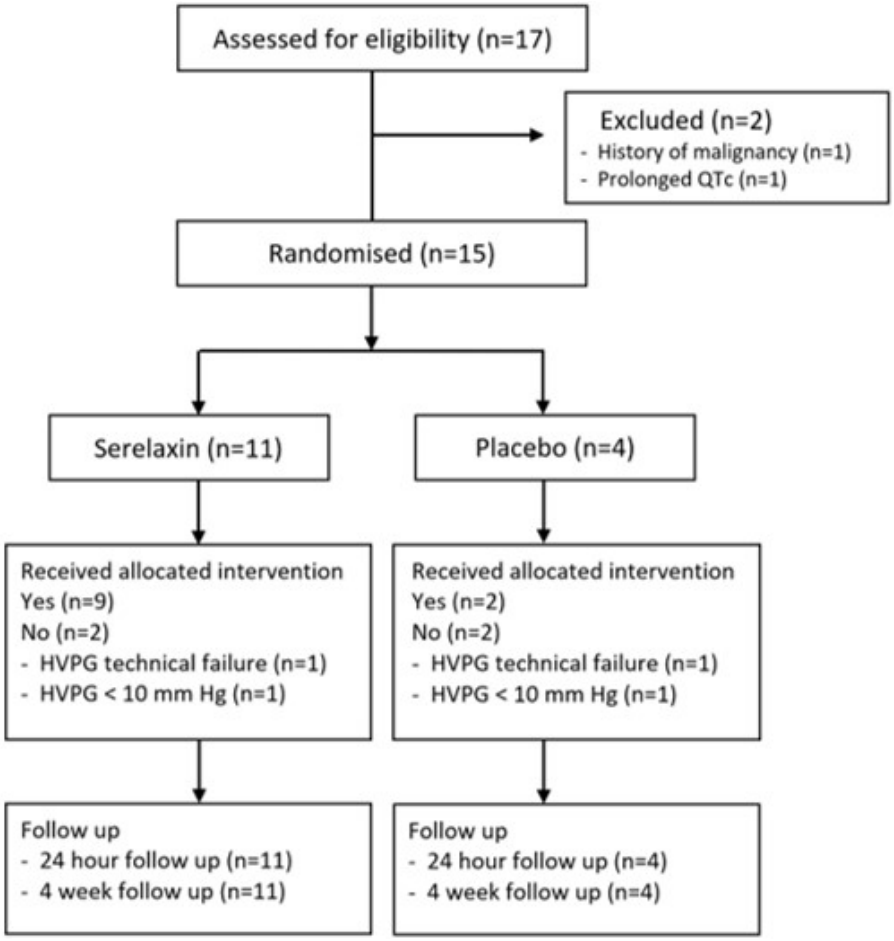
11g. Validation and QC

A random selection of unique analysis and summary tables was QC'd using manual methods (i.e. comparison of results in the table to results calculated by a calculator, spreadsheet, database output or any alternative summarisation tool).

12. Results

12a. Participant Flow (Consort) diagram

Subject disposition is shown in the Consort diagram below. A total of 17 participants were screened. Of these, 2 had a screening failure and did not proceed to randomisation. Fifteen patients were randomised and 11 completed the trial (n=9 serelaxin, n=2 placebo). Reasons for withdrawal were baseline HVPG <10 mmHg (n=2) and technical failure (n=2).



The trial screened 17 participants. Of these participants, 2 had a screening failure and did not proceed to randomisation. Details of these screen failures are provided below.

Study ID	Treatment	Investigator's decision - Please specify
013	Not randomised	Screening failure - historical Squamous Cell carcinoma. Treated and cleared however fulfills exclusion criteria.
017	Not randomised	Screening failure - prolonged QTc

The results in this report are based on the 15 participants who were randomised into the study, unless otherwise specified.

Of the 15 participants who were randomised the following table shows a breakdown of treatment allocations and trial completion.

	Treatment						All	
	Not randomised		Placebo		Serelaxin			
	N	%	N	%	N	%	N	%
Total	2	100	4	100	11	100	17	100
Did the participant withdraw?								
No	0	0	2	50	9	82	11	65
Yes	2	100	2	50	2	18	6	35

12b. Losses and exclusions

The four participants who withdrew following randomisation did not receive any study drug and are not included in any primary or secondary analysis. The reasons for withdrawal are below:

Study ID	Treatment	Other reason for withdrawal - Please specify
009	Serelaxin	Unable to position balloon tipped catheter into hepatic vein therefore procedure had to be abandoned. Follow up phone call at 24 hours/4 weeks continued for safety.
010	Placebo	Unable to position balloon tipped catheter into hepatic vein therefore procedure had to be abandoned. Follow up phone call at 24 hours/4 weeks continued for safety.
011	Serelaxin	HVPG < 10 therefore unable to proceed
015	Placebo	HVPG <10 therefore unable to proceed with study as per protocol

12c. Baseline Data

Baseline participant data is tabulated below. Median age was 56 (range 43-69) and 73% were male. Cirrhosis aetiologies were alcohol (n=10), non-alcoholic fatty liver disease (n=2), hepatitis C (n=2) and hepatitis B (n=1). Subjects were Child-Pugh class A (60%) and B (40%) with median MELD score of 10 (range 6-14). Mean baseline HVPG was 16.3 mmHg (range 10.3-21.7).

- **Participant demographics**

Variable name	Study arm	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Age (years)	Placebo	4	0	59.6	4.1	54.1	56.7	60.2	62.5	63.8	59.5
	Serelaxin	11	0	56.0	7.8	43.2	50.5	56.5	62.4	69.6	55.5
	Total	15	0	56.9	7.0	43.2	51.3	58.0	62.4	69.6	56.5

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Sex						
Male	3	75	8	73	11	73
Female	1	25	3	27	4	27
Ethnicity						
White	4	100	11	100	15	100

- **Physical measurements**

Physical measurements	Study arm	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
BMI	Placebo	4	0	26.2	5.0	19.8	22.6	26.5	29.7	31.8	25.8
	Serelaxin	11	0	29.6	4.4	24.1	26.5	28.0	35.1	36.6	29.3
	Total	15	0	28.7	4.6	19.8	25.7	27.6	31.8	36.6	28.3
Height (cm)	Placebo	4	0	175.1	13.6	155.2	166.6	179.5	183.5	186.0	174.6
	Serelaxin	11	0	168.4	3.7	162.7	166.0	168.0	169.3	175.0	168.3
	Total	15	0	170.2	7.7	155.2	166.0	168.0	175.0	186.0	170.0
Weight (kg)	Placebo	4	0	80.3	19.4	62.6	64.6	77.3	96.1	104.2	78.6
	Serelaxin	11	0	83.8	12.5	68.5	73.9	82.1	99.2	104.9	83.0
	Total	15	0	82.9	13.9	62.6	71.6	82.1	99.2	104.9	81.8

- **Past medical history (PMH): liver disease aetiology and complications**

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Liver disease ongoing						
Yes	4	100	11	100	15	100
Alcohol						
No	2	50	3	27	5	33
Yes	2	50	8	73	10	67
Hepatitis C						
No	3	75	10	91	13	87
Yes	1	25	1	9	2	13
Hepatitis B						
No	3	75	11	100	14	93
Yes	1	25	0	0	1	7
NAFLD						
No	3	75	10	91	13	87
Yes	1	25	1	9	2	13
Autoimmune hepatitis						
No	4	100	11	100	15	100
Primary sclerosing cholangitis						
No	4	100	11	100	15	100
Primary biliary cirrhosis						
No	4	100	11	100	15	100
Haemachromatosis						
No	4	100	11	100	15	100
Cryptogenic						
No	4	100	10	91	14	93
Yes	0	0	1	9	1	7
Other						
No	4	100	11	100	15	100

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Any current/previous liver related complications?						
No	2	50	4	36	6	40
Yes	2	50	7	64	9	60

If yes to `Any current/previous liver related complications`	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	2	100	7	100	9	100
Ascites						
No	0	0	1	14	1	11
Yes	2	100	6	86	8	89
Spontaneous Bacterial Peritonitis						
No	2	100	7	100	9	100
Encephalopathy						
No	1	50	4	57	5	56
Yes	1	50	3	43	4	44
Variceal bleeding						
No	2	100	2	29	4	44
Yes	0	0	5	71	5	56
Other						
No	2	100	7	100	9	100

- **PMH: Non-drug therapies:**

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Any non-drug therapies performed within 30 days of screening?						
No	4	100	11	100	15	100

- **PMH: Current/previous cardiovascular related complications**

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Any current/previous cardiovascular related complications?						
No	4	100	10	91	14	93
Yes	0	0	1	9	1	7

If yes to 'Any current/previous cardiovascular related complications'	Treatment		All	
	Serelaxin			
	N	%	N	%
Total	1	100	1	100
Myocardial Infarction				
No	1	100	1	100
Cerebrovascular Accident				
Yes	1	100	1	100
Other				
No	1	100	1	100

- **PMH: Current/previous other conditions**

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Any current/previous other conditions?						
No	0	0	1	9	1	7
Yes	4	100	10	91	14	93

If yes to 'Any current/previous other conditions'	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	10	100	14	100
Asthma						
No	3	75	7	70	10	71
Yes	1	25	3	30	4	29
COPD						
No	4	100	10	100	14	100
Diabetes						
No	4	100	7	70	11	79
Yes	0	0	3	30	3	21
Inflammatory Bowel Disease						
No	4	100	10	100	14	100
Epilepsy/Seizures						
No	4	100	9	90	13	93
Yes	0	0	1	10	1	7
Haematological disorder						
No	4	100	7	70	11	79
Yes	0	0	3	30	3	21
Dermatological disorder						
No	2	50	7	70	9	64
Yes	2	50	3	30	5	36

- **PMH: Additional significant illnesses**

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Additional significant illnesses						
No	1	25	4	36	5	33
Yes	3	75	7	64	10	67

If yes to `Additional significant illnesses`	Treatment				All	
	Placebo		Serelaxin		All	
	N	%	N	%	N	%
Total	3	100	7	100	10	100
Illness 1						
Cholecystectomy	0	0	1	14	1	10
Depression	1	33	0	0	1	10
Fracture subluxation right ankle	0	0	1	14	1	10
Hypertension	1	33	2	29	3	30
Peripheral neuropathy	0	0	2	29	2	20
Retinal haemorrhage	1	33	0	0	1	10
Right knee injury - bomb blast in army 1997	0	0	1	14	1	10
Illness 2						
Carpel tunnel syndrome	0	0	1	14	1	10
Depression	0	0	1	14	1	10
Duodenal ulcer	1	33	0	0	1	10
Osteopenia	1	33	1	14	2	20
Post traumatic stress disorder	0	0	1	14	1	10
Renal Calculi	1	33	0	0	1	10
Right lateral tongue - hyperkeratosis, inflammation	0	0	1	14	1	10
Unstable Angina	0	0	1	14	1	10
fracture right little finger metacarpal	0	0	1	14	1	10

If yes to `Additional significant illnesses`	Treatment				All	
	Placebo		Serelaxin		All	
	N	%	N	%	N	%
Illness 3						
3rd illness not recorded	0	0	4	57	4	40
Colonic polyps	1	33	0	0	1	10
Depression	0	0	1	14	1	10
Glaucoma	0	0	1	14	1	10
Hiatus Henia	1	33	0	0	1	10
Leishmaniasis	0	0	1	14	1	10
Sciatica	1	33	0	0	1	10
Illness 4						
4th illness not recorded	0	0	5	71	5	50
Back pain	0	0	1	14	1	10
Chronic bronchitis	1	33	0	0	1	10
Hypertension	0	0	1	14	1	10
Raynauds	1	33	0	0	1	10
pneumothorax	1	33	0	0	1	10
Illness 5						
5th illness not recorded	1	33	6	86	7	70
Depression	1	33	0	0	1	10
Osteoarthritis	1	33	0	0	1	10
Vaginal prolapse	0	0	1	14	1	10

12d. Alcohol consumption

	Treatment				All	
	Placebo		Serelaxin		All	
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Is alcohol being consumed?						
No	3	75	8	73	11	73
Yes	1	25	3	27	4	27

If yes to 'Is alcohol being consumed?'	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	1	100	3	100	4	100
What is the average weekly intake						
<1 unit/week	1	100	3	100	4	100

If no to 'Is alcohol being consumed?'	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	3	100	8	100	11	100
Participant abstinent for:						
6-12 months	1	33	2	25	3	27
1-2 years	0	0	2	25	2	18
>2 years	2	67	4	50	6	55

12e. Medications within 30 days of screening

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Current/recent medications						
Yes	4	100	11	100	15	100
If yes, has the participant recently enrolled/is currently enrolled in another clinical trial?						
No	3	75	6	55	9	60
Yes	1	25	5	45	6	40

12f. Physical examination

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Was a physical examination performed?						
Yes	4	100	11	100	15	100

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
General appearance						
Normal	4	100	11	100	15	100
Skin						
Abnormal	3	75	10	91	13	87
Normal	1	25	1	9	2	13
Eyes, Ears, Nose and Throat						
Abnormal	0	0	1	9	1	7
Normal	4	100	10	91	14	93
Head, Neck and Thyroid						
Normal	4	100	11	100	15	100
Cardiovascular						
Abnormal	0	0	1	9	1	7
Normal	4	100	10	91	14	93
Respiratory						
Normal	4	100	11	100	15	100
Abdomen						
Abnormal	3	75	5	45	8	53
Normal	1	25	6	55	7	47
Extremities						
Abnormal	0	0	2	18	2	13

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Extremities						
Normal	4	100	9	82	13	87
Musculoskeletal						
Normal	4	100	11	100	15	100
Neurological						
Abnormal	0	0	1	9	1	7
Normal	4	100	10	91	14	93

Skin abnormalities	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	3	100	10	100	13	100
If abnormal...						
Clinically significant	0	0	1	10	1	8
Not clinically significant	3	100	9	90	12	92

Abdomen abnormalities	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	3	100	5	100	8	100
If abnormal...						
Not clinically significant	3	100	5	100	8	100

Eyes, ears, nose and throat abnormalities	Treatment		All	
	Serelaxin			
	N	%	N	%
Total	1	100	1	100
If abnormal...				
Not clinically significant	1	100	1	100

Extremeties abnormalities	Treatment		All	
	Serelaxin			
	N	%	N	%
Total	2	100	2	100
If abnormal...				
Not clinically significant	2	100	2	100

Cardiovascular abnormalities	Treatment		All	
	Serelaxin			
	N	%	N	%
Total	1	100	1	100
If abnormal...				
Not clinically significant	1	100	1	100

Neurological abnormalities	Treatment		All	
	Serelaxin			
	N	%	N	%
Total	1	100	1	100
If abnormal...				
Not clinically significant	1	100	1	100

12g. Vital signs

Vital signs	Study arm	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Diastolic BP	Placebo	4	0	78.3	10.8	66.3	71.7	77.0	84.8	92.7	77.7
	Serelaxin	11	0	80.8	11.2	65.3	69.7	78.7	91.7	95.3	80.0
	Total	15	0	80.1	10.7	65.3	69.7	77.0	91.7	95.3	79.4
Heart rate (bpm)	Placebo	4	0	68.8	2.4	66.3	66.7	68.8	70.8	71.0	68.7
	Serelaxin	11	0	74.7	16.6	46.3	62.3	73.0	94.0	97.0	72.9
	Total	15	0	73.1	14.4	46.3	66.3	70.7	81.0	97.0	71.8
Respiratory rate	Placebo	4	0	15.5	1.9	14.0	14.0	15.0	17.0	18.0	15.4
	Serelaxin	11	0	14.4	2.3	12.0	12.0	16.0	16.0	18.0	14.2
	Total	15	0	14.7	2.2	12.0	12.0	16.0	16.0	18.0	14.5
Systolic BP	Placebo	4	0	132.3	16.0	111.7	120.5	134.0	144.0	149.3	131.5
	Serelaxin	11	0	150.0	16.3	126.0	132.3	155.3	163.7	173.3	149.1
	Total	15	0	145.3	17.6	111.7	129.3	145.0	156.0	173.3	144.2

12h. Electrocardiography (ECG)

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Was an ECG performed?						
Yes	4	100	11	100	15	100
Was a second ECG performed?						
Yes	4	100	11	100	15	100
Was a third ECG performed?						
Yes	4	100	11	100	15	100

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
If yes, was the ECG...						
Normal	4	100	8	73	12	80
Abnormal but not clinically significant	0	0	3	27	3	20
If yes, was ECG 2...						
Normal	4	100	9	82	13	87
Abnormal but not clinically significant	0	0	2	18	2	13
If yes, was ECG 3...						
Normal	4	100	8	73	12	80
Abnormal but not clinically significant	0	0	3	27	3	20

12i. Child-Pugh and MELD scores

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
Child Pugh Score						
5	2	50	3	27	5	33
6	1	25	3	27	4	27
7	1	25	3	27	4	27
9	0	0	2	18	2	13

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
MELD score						
6	1	25	0	0	1	7
7	1	25	0	0	1	7
8	1	25	1	9	2	13
9	0	0	3	27	3	20
10	0	0	1	9	1	7
11	1	25	2	18	3	20
12	0	0	2	18	2	13
13	0	0	1	9	1	7
14	0	0	1	9	1	7

12j. Pulse wave velocity (m/s)

Variable name	Study arm	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Pulse wave velocity	Placebo	4	0	9.2	1.9	8.0	8.0	8.5	10.4	11.9	9.0
	Serelaxin	11	0	8.4	1.5	6.0	7.3	8.9	9.9	10.2	8.3
	Total	15	0	8.6	1.6	6.0	7.4	8.9	9.9	11.9	8.5
AIx at aortic	Placebo	4	0	48.8	11.6	35.1	40.4	48.7	57.3	62.8	47.7
	Serelaxin	11	0	36.0	6.5	28.0	29.2	36.8	43.5	45.0	35.4
	Total	15	0	39.4	9.7	28.0	30.2	38.0	45.0	62.8	38.3
AIx at brachial*	Placebo	4	0	22.1	22.8	-5.1	5.4	21.9	38.7	49.6	.
	Serelaxin	11	0	-3.2	12.9	-19.1	-16.8	-1.7	11.6	14.6	.
	Total	15	0	3.5	19.1	-19.1	-14.8	0.8	14.6	49.6	.
Arterial age	Placebo	4	0	50.3	9.3	42.0	42.5	49.0	58.0	61.0	49.6
	Serelaxin	11	0	45.7	16.8	15.5	33.0	55.5	61.0	61.0	42.1
	Total	15	0	46.9	15.0	15.5	35.5	55.0	61.0	61.0	44.0
Brachial Diastolic BP	Placebo	4	0	83.4	13.2	75.5	75.8	77.5	91.0	103.0	82.7
	Serelaxin	11	0	80.9	11.3	62.5	71.0	81.0	91.0	101.0	80.1
	Total	15	0	81.6	11.4	62.5	75.5	79.0	91.0	103.0	80.8
Brachial MAP	Placebo	4	0	99.3	13.6	89.5	89.8	94.5	108.8	118.5	98.6
	Serelaxin	11	0	100.6	11.8	82.0	91.0	99.5	110.0	122.0	99.9
	Total	15	0	100.2	11.8	82.0	90.0	99.0	110.0	122.0	99.6
Brachial PP	Placebo	4	0	47.4	9.3	40.5	41.5	44.0	53.3	61.0	46.7
	Serelaxin	11	0	58.9	11.6	42.0	47.0	63.0	69.0	75.0	57.7
	Total	15	0	55.8	11.9	40.5	45.0	57.5	66.5	75.0	54.6
Brachial Systolic BP	Placebo	4	0	130.8	16.0	116.5	117.3	129.0	144.3	148.5	130.0
	Serelaxin	11	0	139.8	16.1	119.0	128.0	138.0	160.0	164.0	139.0
	Total	15	0	137.4	16.0	116.5	120.0	138.0	148.5	164.0	136.5
Central PP Ao	Placebo	4	0	52.7	10.9	43.7	45.0	49.6	60.5	68.0	51.0
	Serelaxin	11	0	60.6	12.4	42.4	47.5	63.5	70.2	76.9	59.0
	Total	15	0	58.5	12.2	42.4	46.3	62.1	68.0	76.9	56.7
Central SBPao	Placebo	4	0	136.1	18.1	119.2	120.8	134.7	151.5	155.9	135.2
	Serelaxin	11	0	141.5	16.2	115.6	126.4	139.6	155.4	168.8	140.6
	Total	15	0	140.0	16.2	115.6	126.0	139.6	155.4	168.8	139.1
Ejection duration	Placebo	4	0	336.9	33.3	290.0	313.8	346.3	360.0	365.0	335.6
	Serelaxin	11	0	323.6	26.7	285.0	300.0	322.5	347.5	367.5	322.6
	Total	15	0	327.2	28.0	285.0	300.0	330.0	355.0	367.5	326.0

* Geometric mean has not been calculated for AIx at brachial because it is possible for this measurement to be negative as well as positive.

12k. Impedance cardiography

Haemodynamic measurements	Study arm	N	N missing	Geometric mean	Mean	St.Dev	Min	Q1	Median	Q3	Max
Cardiac Index	Placebo	4	0	3.2	3.3	0.4	2.8	3.0	3.2	3.6	3.8
	Serelaxin	11	0	3.9	3.9	0.5	3.0	3.4	4.0	4.3	4.8
	Total	15	0	3.7	3.7	0.6	2.8	3.3	3.8	4.1	4.8
IVCP	Placebo	4	0	6.4	7.4	3.7	2.3	4.8	8.2	10.0	11.0
	Serelaxin	10	1	7.3	8.3	3.1	0.3	8.0	8.7	10.0	11.0
	Total	14	1	7.0	8.0	3.2	0.3	7.3	8.7	10.0	11.0
MAP	Placebo	4	0	99.5	100.5	16.9	86.3	87.3	96.5	113.7	122.7
	Serelaxin	11	0	93.4	94.0	10.9	80.3	82.0	96.3	100.3	111.7
	Total	15	0	95.0	95.7	12.4	80.3	84.7	96.3	104.7	122.7
SVRI	Placebo	4	0	2260	2338	727.4	1703	1813	2152	2862	3343
	Serelaxin	11	0	1701	1735	363.7	1344	1369	1680	2146	2317
	Total	15	0	1835	1896	532.8	1344	1455	1794	2169	3343

12l. Primary Outcome

The primary outcome is to examine if the baseline to 2 hr change in fasting hepatic venous pressure gradient (HVPG) is a clinically significant one.

In those allocated to the serelaxin arm (n=11), two participants were withdrawn. For one of these participants it was not possible to obtain a HVPG measurement and in the other their HVPG was <10 making them unable to continue in the study. These two participants shall not be included in any further analyses, leaving 9 participants.

The following tables present the descriptive statistics for the change and the results from a paired-samples t-test using both the arithmetic mean and the geometric mean.

Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
HVPG	Baseline: 1 h	9	0	15.9	3.3	10.3	14.7	16.7	18.7	19.3	15.6
	Start of Infusion +2hr	9	0	15.6	4.3	10.7	12.3	14.7	19.0	23.3	15.1

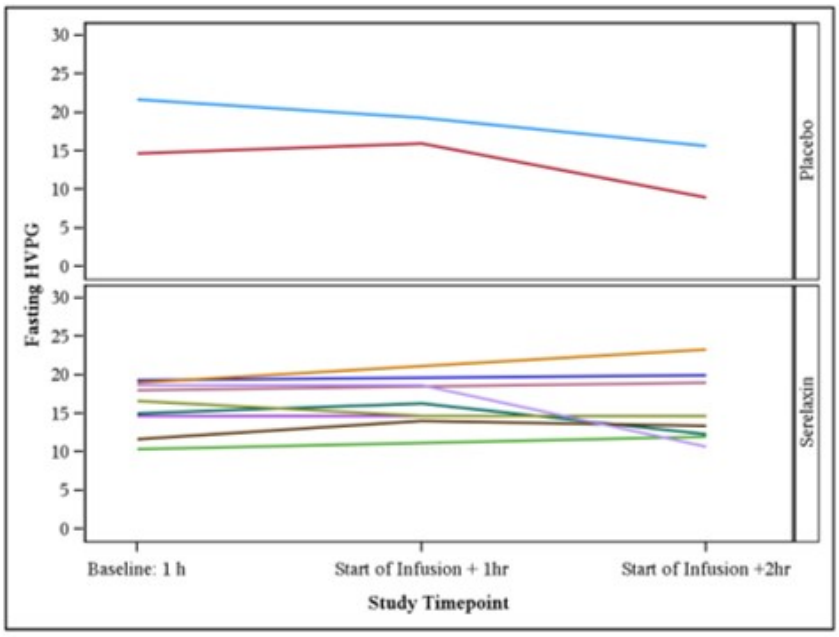
The results from the paired t-tests show that there is no evidence of a significant change between baseline and 2 hr change in fasting HPVG ($p\text{-value}_{arithmetic}=0.76$; $p\text{-value}_{geometric}=0.68$).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion2h)	9	0.4	3.5	-2.3	3.1	0.76

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion2h)	9	1.0	0.2	0.9	1.2	0.68

The following is a graphical presentation of fasting HVPG over the duration of infusion by treatment allocation. Each line represents an individual participant.

Fasting hepatic venous pressure gradient



12m. Secondary Outcomes

- **Change in fasting HVPG after 1 hr from baseline**

This analysis examines the change from baseline in fasting HVPG after 1 hr serelaxin infusion. The table below presents the descriptive statistics in those treated with serelaxin at baseline and at 1 hr after infusion. Note that 5 participants did not have HVPG measurements taken one after the start of their infusion. This was due to a decision by the study team to focus efforts on the 2 hr HVPG measurement.

Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
HVPG	Baseline: 1 h	9	0	15.9	3.3	10.3	14.7	16.7	18.7	19.3	15.6
	Start of Infusion + 1hr	4	5	15.9	2.1	14.0	14.3	15.5	17.5	18.7	15.8

The following tables present the results from paired t-tests using both arithmetic and geometric means. The results from the paired t-tests show that there is no evidence of a significant change between baseline and 1 hr change in fasting HPVG ($p\text{-value}_{arithmetic}$=0.69; $p\text{-value}_{geometric}$=0.63).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion1h)	4	-0.4	1.9	-3.4	2.6	0.69

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion1h)	4	1.0	0.1	0.8	1.2	0.63

- **Change in hepatic blood flow**

The following table shows the descriptive statistics for hepatic blood flow pre and post infusion in those participants who received serelaxin:

Time point	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Baseline 1 hr	7	4	1.5	0.8	0.4	0.8	1.5	2.2	2.7	1.2
Start of infusion + 2 hr	5	6	1.2	0.8	0.4	0.6	1.1	1.3	2.4	1.0

The following table shows the result of a paired t-test on the baseline to start of infusion + 2 hr change (post minus pre, so that a negative value indicates a drop in measurements) and from this it can be seen that there is no evidence of a statistically significant change using either the arithmetic mean p=0.1451 or geometric mean p=0.1465.

Arithmetic mean:

N	Mean	95% CL Mean		Std Dev	DF	t Value	Pr > |t|
5	-0.2781	-0.7054	0.1493	0.3442	4	-1.81	0.1451

Geometric mean:

N	Mean	95% CL Mean		Coefficient of Variation	DF	t Value	Pr > |t|
5	0.8432	0.6481	1.0971	0.2144	4	-1.80	0.1465

- **Change in Hepatic Sinusoidal Resistance**

The following table shows the descriptive statistics for hepatic sinusoidal resistance pre and post infusion in those participants who received serelaxin.

Time point	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Baseline 1 hr	7	4	1160	649.2	343.3	615.3	1039	1860	2176	994.9
Start of infusion + 2 hr	5	6	1187	721.3	444.6	891.7	922.8	1326	2350	1027

The following table shows the result of a paired t-test on the baseline to start of infusion + 2 hr change (post minus pre, so that a negative value indicates a drop in measurements) and from this it can be seen that there is no evidence of a statistically significant change using either the arithmetic mean p=0.9010 or geometric mean p=0.7124.

Arithmetic mean:

N	Mean	95% CL Mean		Std Dev	DF	t Value	Pr > |t|
5	-19.0299	-417.8	379.7	321.1	4	-0.13	0.9010

Geometric mean:

N	Mean	95% CL Mean		Coefficient of Variation	DF	t Value	Pr > |t|
5	1.0518	0.7381	1.4987	0.2911	4	0.40	0.7124

- **Change in IVCP**

This analysis examines the baseline to 2 hr change in inferior vena cava pressure (IVCP). The table below presents the descriptive statistics in those treated with serelaxin.

Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
IVCP	Baseline: 1 h	8	1	8.2	3.4	0.3	8.0	8.7	10.5	11.0	7.0
	Start of Infusion +2hr	8	1	9.0	2.4	7.0	7.5	7.8	10.3	14.0	8.8

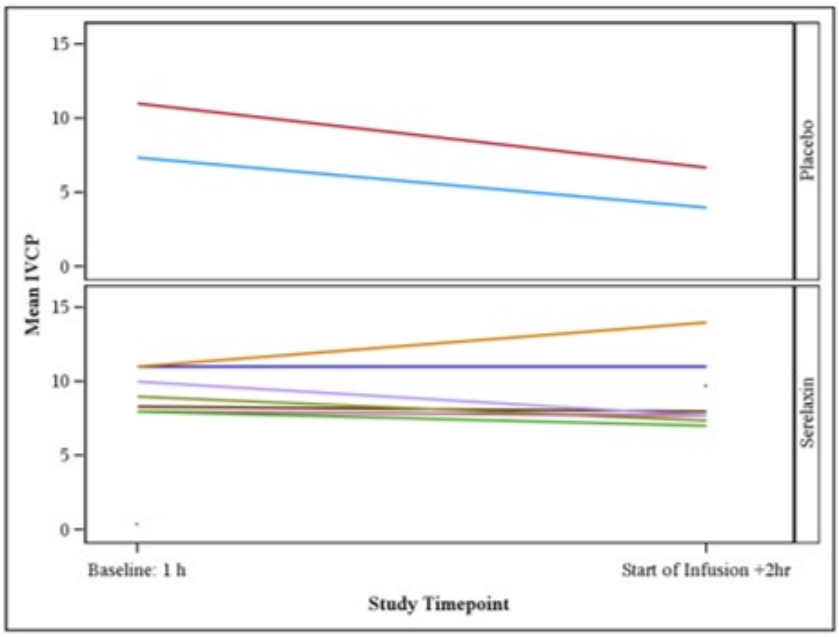
The following tables present the results from paired t-tests using both arithmetic and geometric means. The results from the paired t-tests show that there is no evidence of a significant change between baseline and 2 hr change in IVCP ($p\text{-value}_{arithmetic}=0.58$; $p\text{-value}_{geometric}=0.27$).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion2h)	7	0.4	1.7	-1.2	2.0	0.58

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion2h)	7	1.0	0.1	1.0	1.1	0.27

The following is a graphical presentation of IVCP over the duration of infusion by treatment allocation. Each line represents an individual participant.

Mean inferior vena cava pressure (IVCP)



- **Change in cardiac index**

The table below presents the descriptive statistics of the baseline to 2 hr change of cardiac index in those treated with serelaxin.

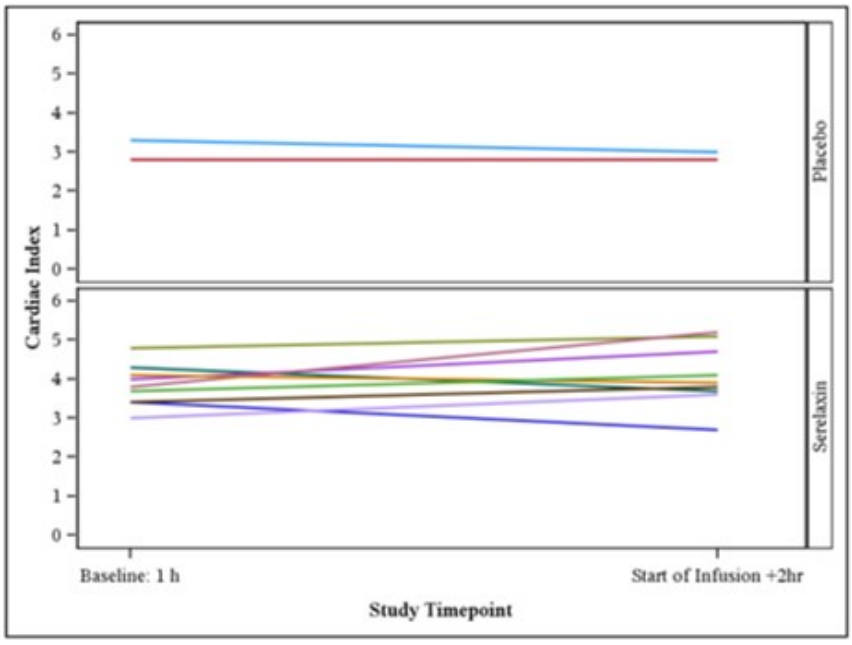
Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Cardiac index	Baseline: 1 h	9	0	3.8	0.5	3.0	3.4	3.8	4.1	4.8	3.8
	Start of Infusion +2hr	9	0	4.1	0.8	2.7	3.7	3.9	4.7	5.2	4.0

The following tables present the results from paired t-tests using both arithmetic and geometric means. The results from the paired t-tests show that there is no evidence of a signficant change between baseline and 2 hr change in cardiac index ($p\text{-value}_{arithmetic}$=0.28; $p\text{-value}_{geometric}$=0.44).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion2h)	9	-0.3	0.7	-0.8	0.3	0.28

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion2h)	9	1.0	0.1	0.9	1.1	0.44

Cardiac index



- **Change in SVRI**

The table below presents the descriptive statistics of the baseline to 2 hr change of systemic vascular resistance index (SVRI) in those treated with serelaxin.

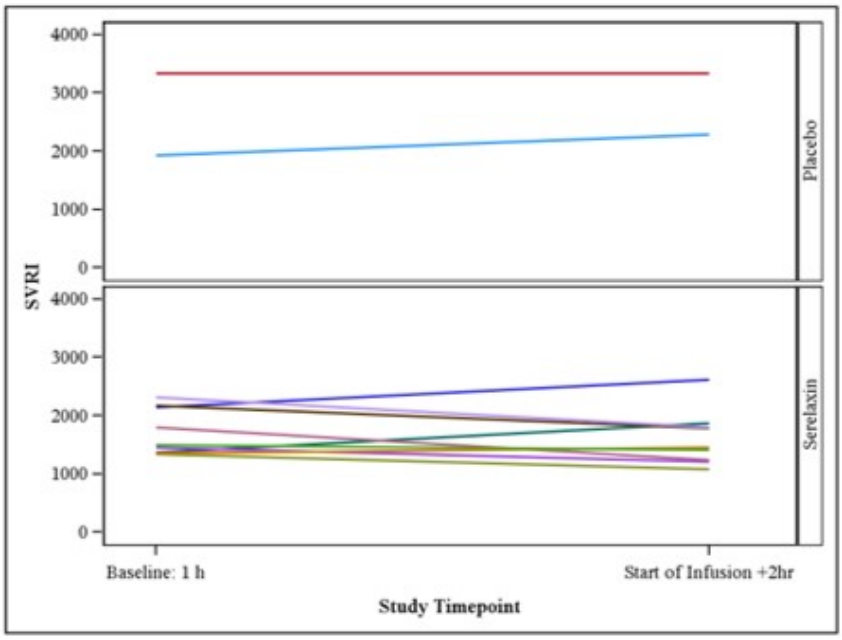
Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
SVRI	Baseline: 1 h	9	0	1716	397.7	1344	1369	1501	2146	2317	1677
	Start of Infusion +2hr	9	0	1605	473.9	1073	1238	1459	1797	2617	1549

The following tables present the results from paired t-tests using both arithmetic and geometric means. The results from the paired t-tests show that there is no evidence of a significant change between baseline and 1 hr change in systemic vascular resistance index ($p\text{-value}_{arithmetic}$=0.42; $p\text{-value}_{geometric}$=0.32).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion2h)	9	111.1	394.4	-192	414.3	0.42

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion2h)	9	1.0	0.0	1.0	1.0	0.32

Superior vascular resistance index (SVRI)



- **Change in aortic pulse wave velocity**

The table below presents the descriptive statistics of the baseline to 2 hr change of aortic pulse wave velocity in those treated with serelaxin.

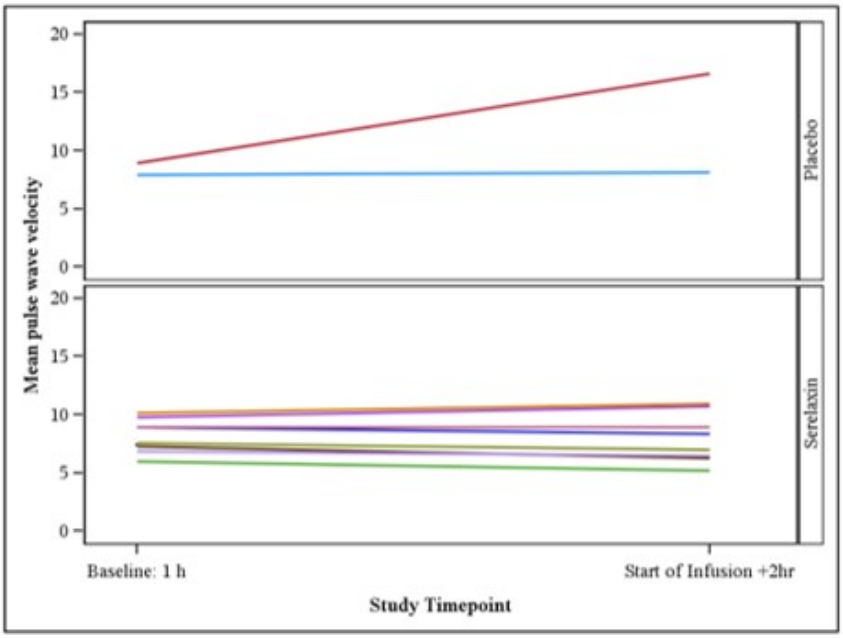
Variable name	Study timepoint	N	N missing	Mean	St.Dev	Min	Q1	Median	Q3	Max	Geometric mean
Pulse wave velocity	Baseline: 1 h	9	0	8.1	1.4	6.0	7.3	7.6	8.9	10.2	8.0
	Start of Infusion +2hr	8	1	8.0	2.1	5.2	6.4	7.7	9.8	11.0	7.7

The following tables present the results from paired t-tests using both arithmetic and geometric means. The results from the paired t-tests show that there is no evidence of a significant change between baseline and 2 hr change in aortic pulse wave velocity ($p\text{-value}_{arithmetic}$=0.49; $p\text{-value}_{geometric}$=0.19).

Test	N	Mean of diff	Std.Dev of diff	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Arithmetic (Baseline-Infusion2h)	8	0.2	0.7	-0.4	0.8	0.49

Test	N	Geometric mean of diff	Coefficient of variation	Lower 95%CI of diff	Upper 95%CI of diff	p-value
Geometric (Baseline/Infusion2h)	8	1.0	0.0	1.0	1.1	0.19

Mean pulse wave velocity



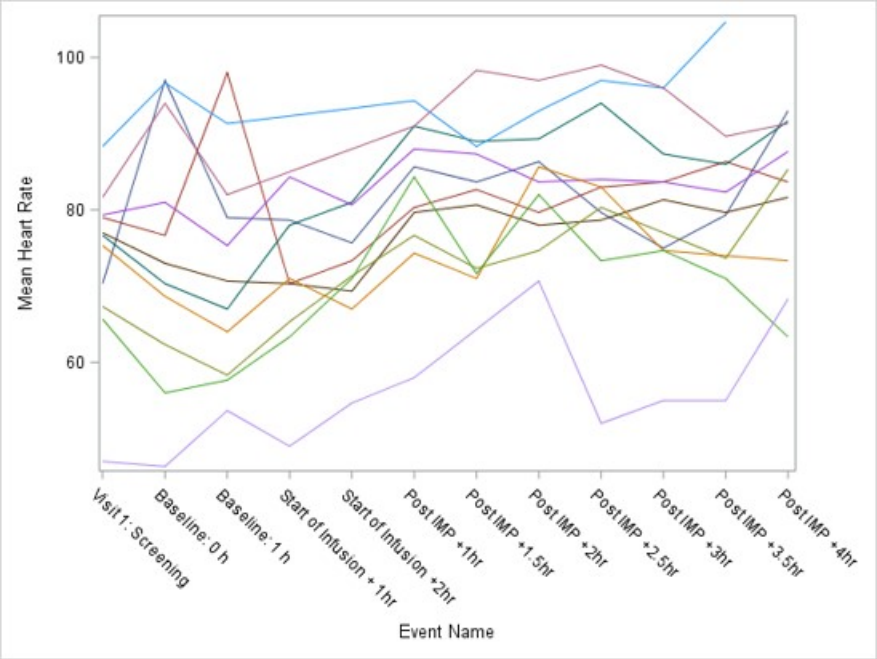
- **Change in heart rate and blood pressure**

The following three plots show the mean values for heart rate, systolic BP, diastolic BP as measured across the study period where the mean is calculated from three readings.

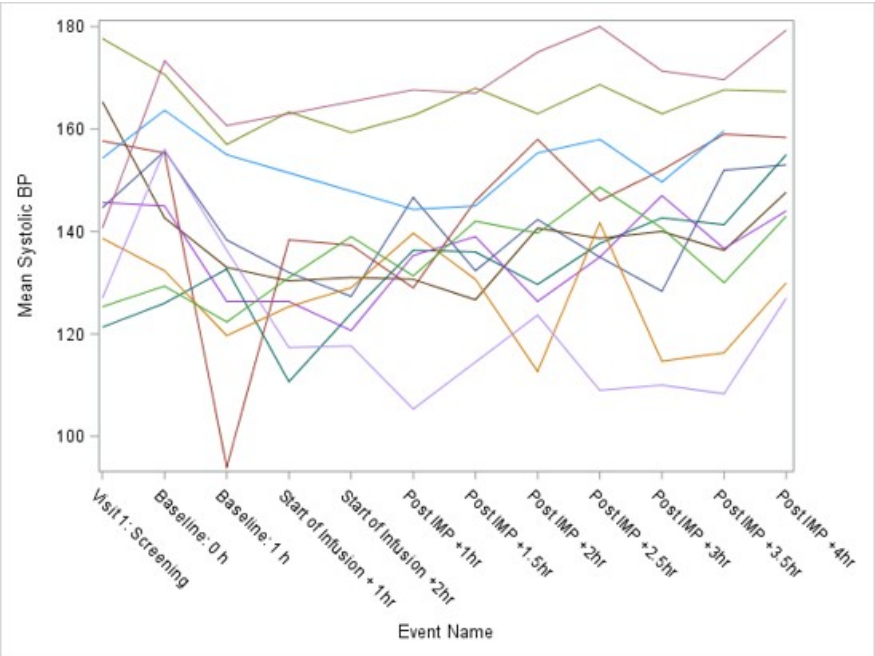
The plots are shown for those treated and each line represents a different participant.

Please note that although the space between time points in this graph are displayed as equidistant the time gap between points may not be equal.

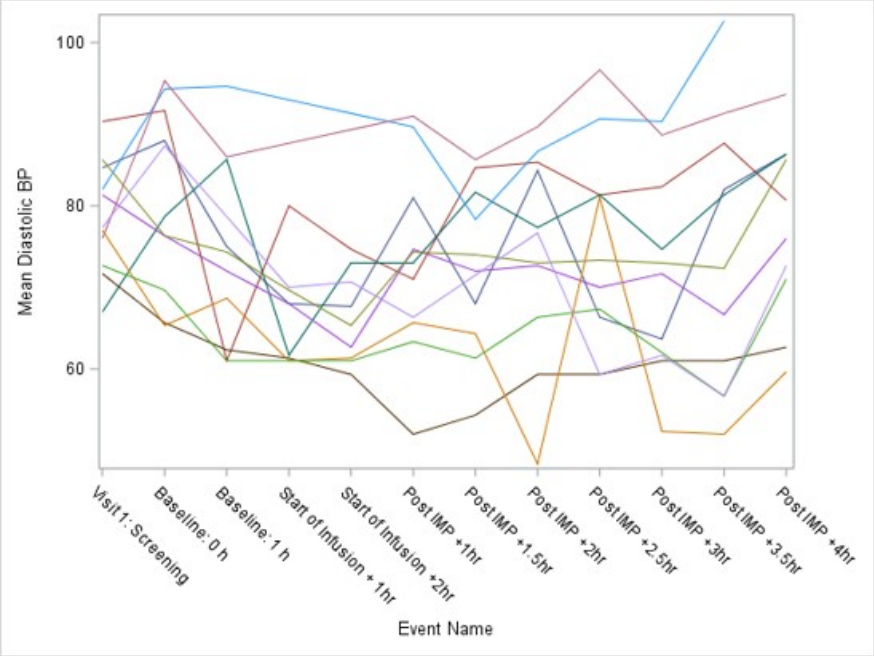
Mean heart rate



Mean systolic blood pressure



Diastolic blood pressure



- **Change in biomarkers**

This analysis not been performed as no biomarkers were measured.

- **Change in HVPG in placebo group**

This analysis is a repeat of the primary outcome analysis but in those allocated to the placebo group. No formal comparison will be made between treatment and placebo. Only two participants have results at both study timepoints and the other two participants in the placebo group were withdrawn. One was withdrawn due to their HVPG measurement being <10 mmHg, and the other was withdrawn as a HVPG measurement could not be taken. Due to the small number of participants in this analysis a paired t-test shall not be performed.

Study ID	Baseline	Infusion+2h
002	14.666666667	9
007	21.666666667	15.666666667

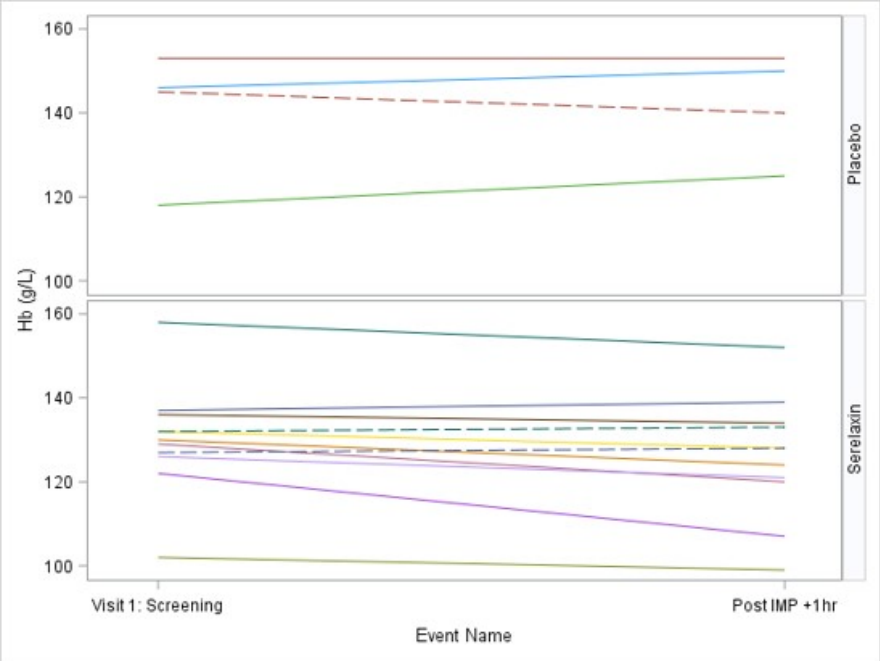
13. Safety

13a. Laboratory blood tests

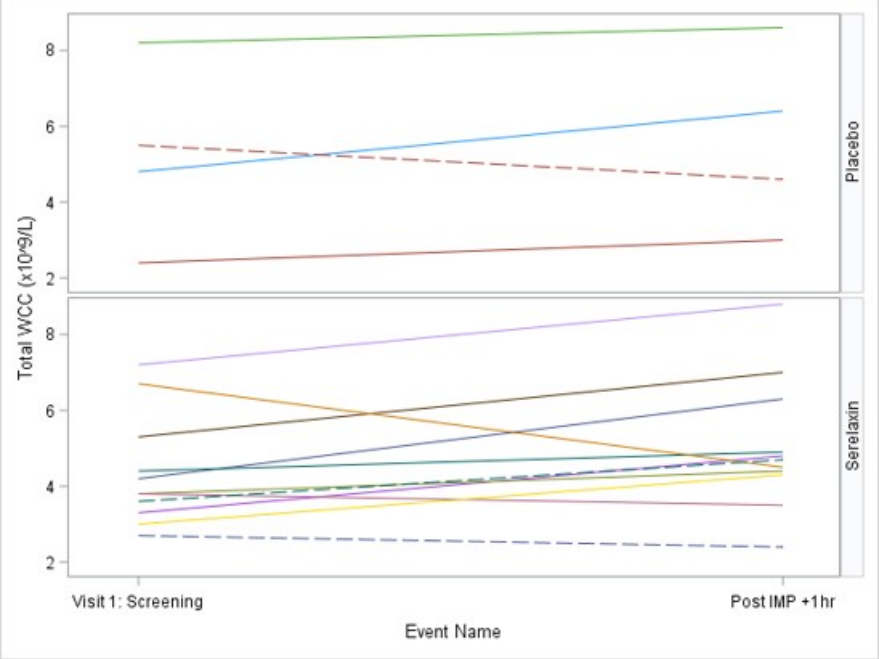
For each of the blood test measurements we have them recorded at screening and again at 1 hr post IMP. The following plots show the screening to post IMP change. Each line represents a participant and each graph contains two panels to present the information broken down by treatment allocation.

Please note eGFR was collected but has not been shown in these plots as all responses were recorded as ≥60 with the exception of the screening result in participant 001 which was 58.

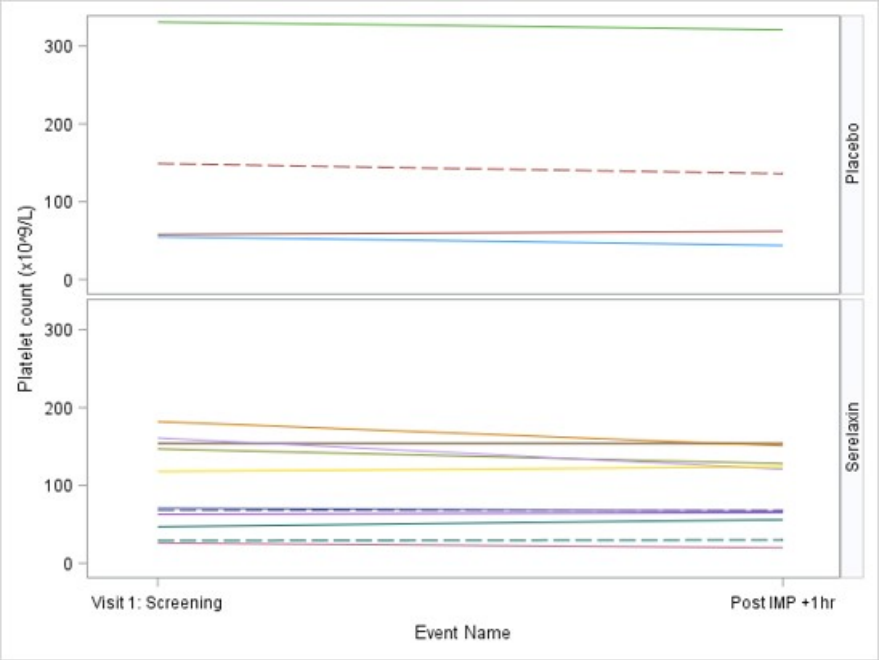
Haemoglobin



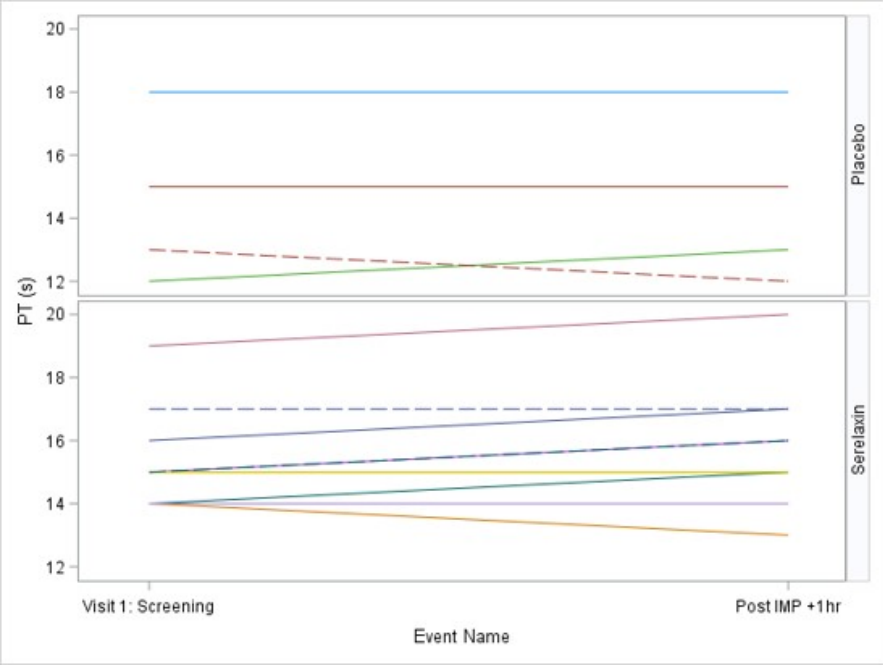
Total white cell count



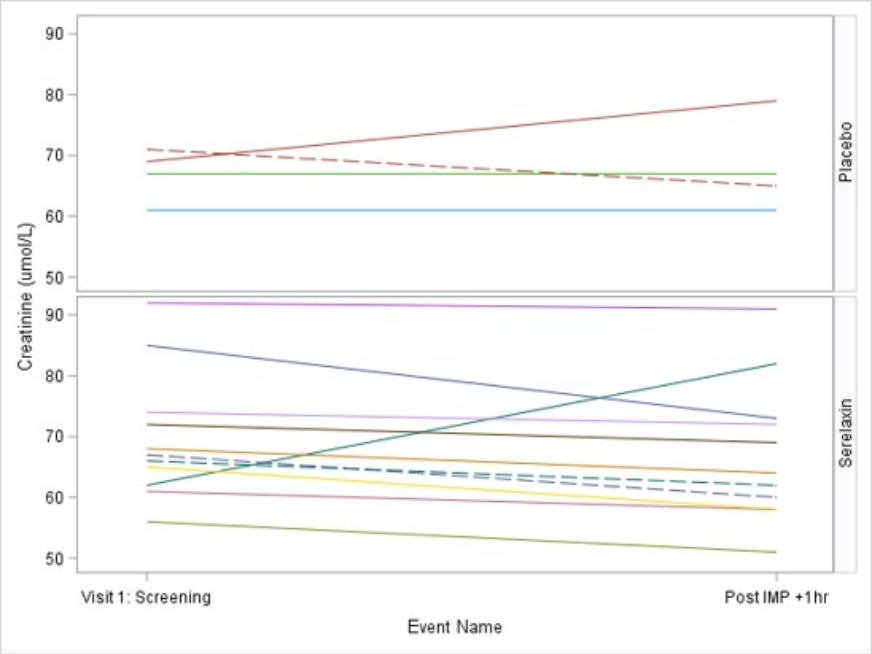
Platelets



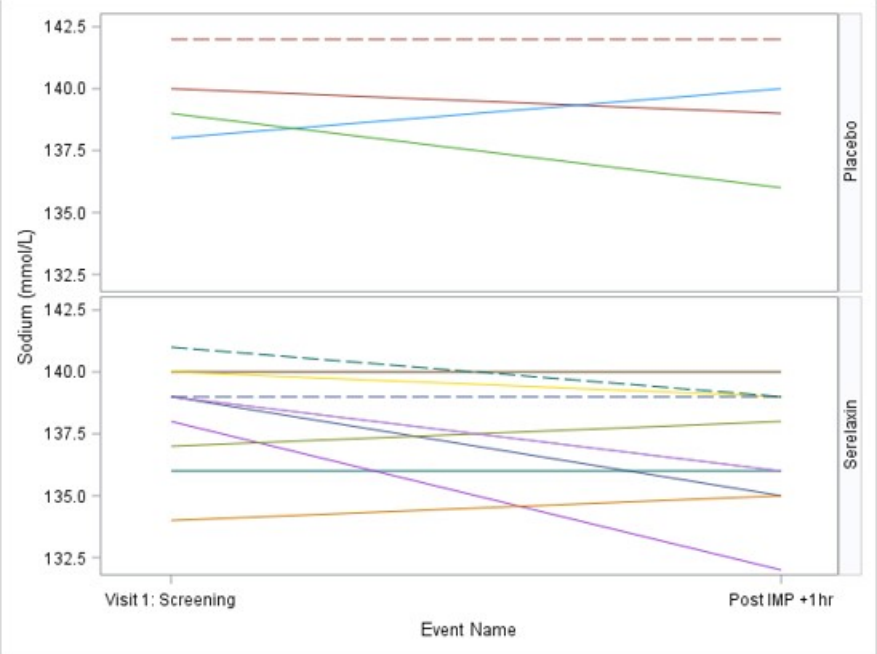
Prothrombin time



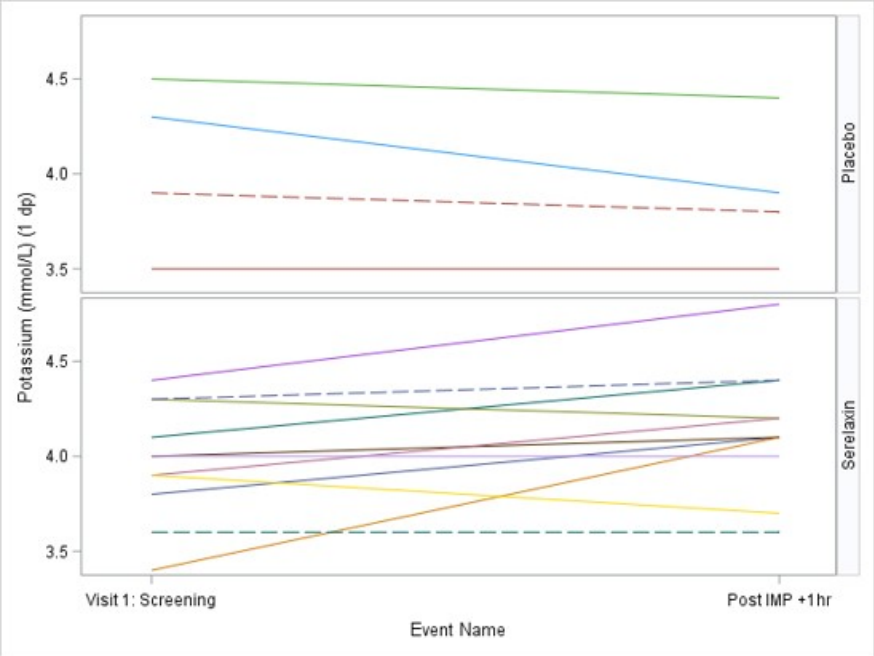
Serum creatinine



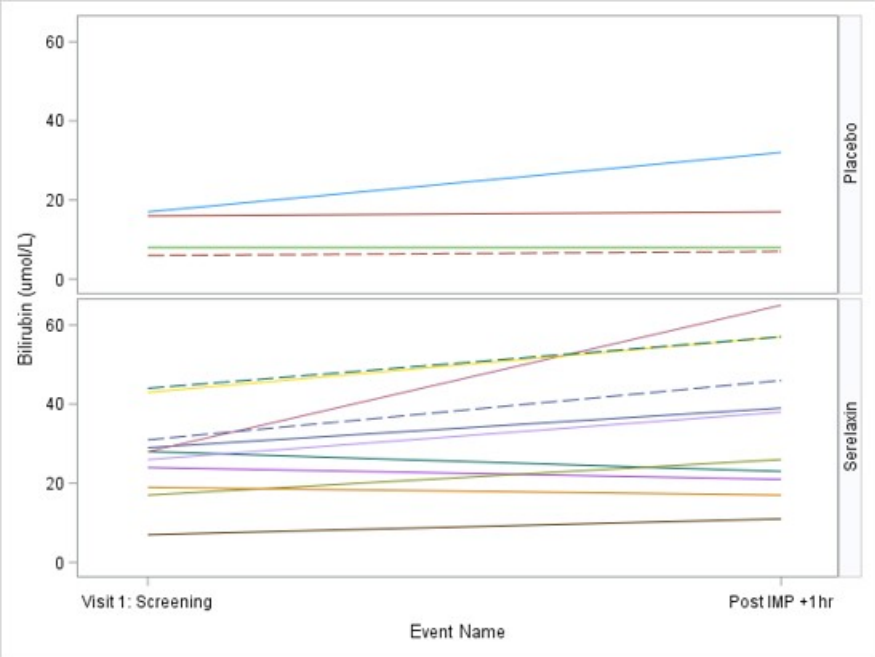
Serum sodium



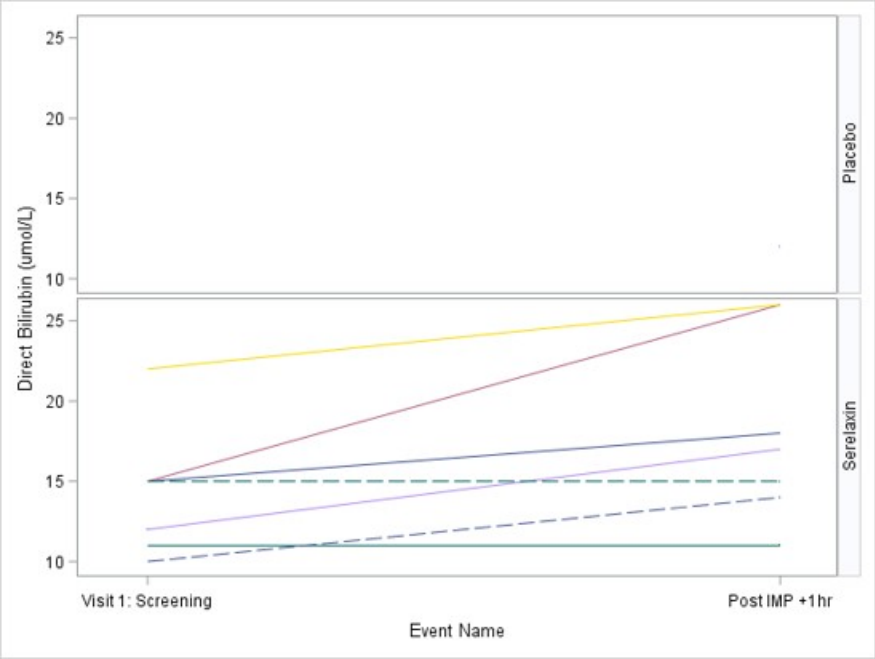
Serum potassium



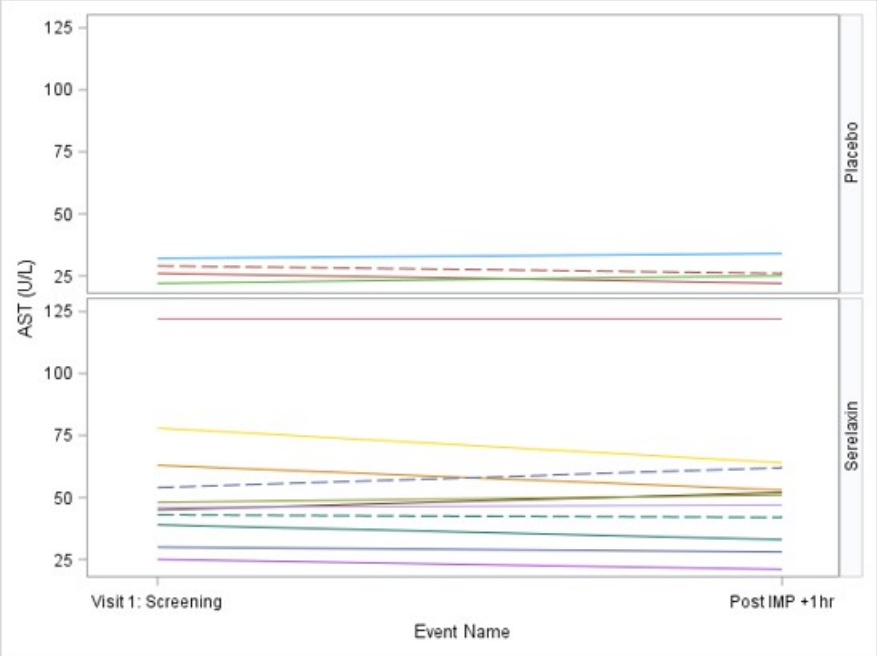
Serum total bilirubin



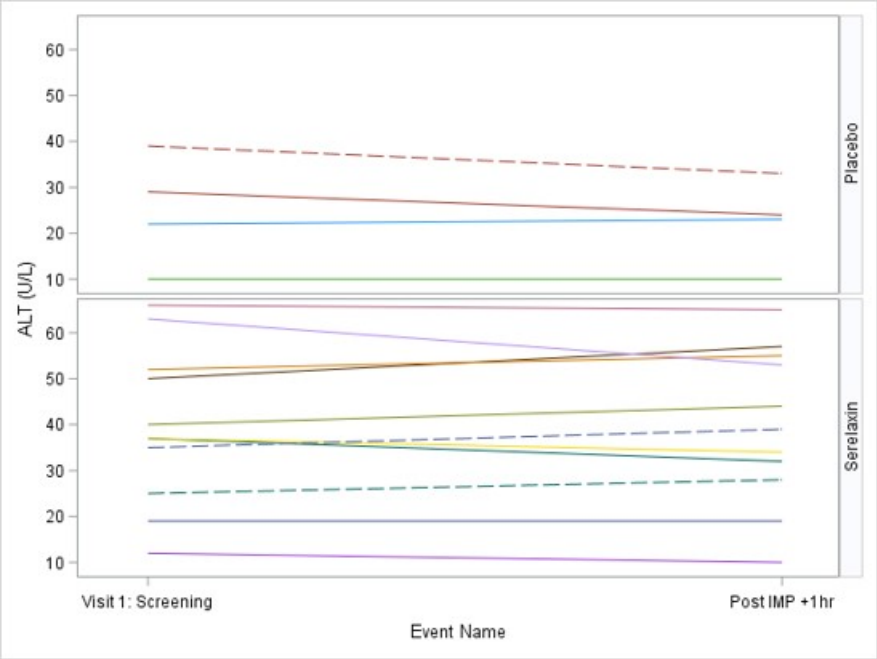
Serum direct bilirubin



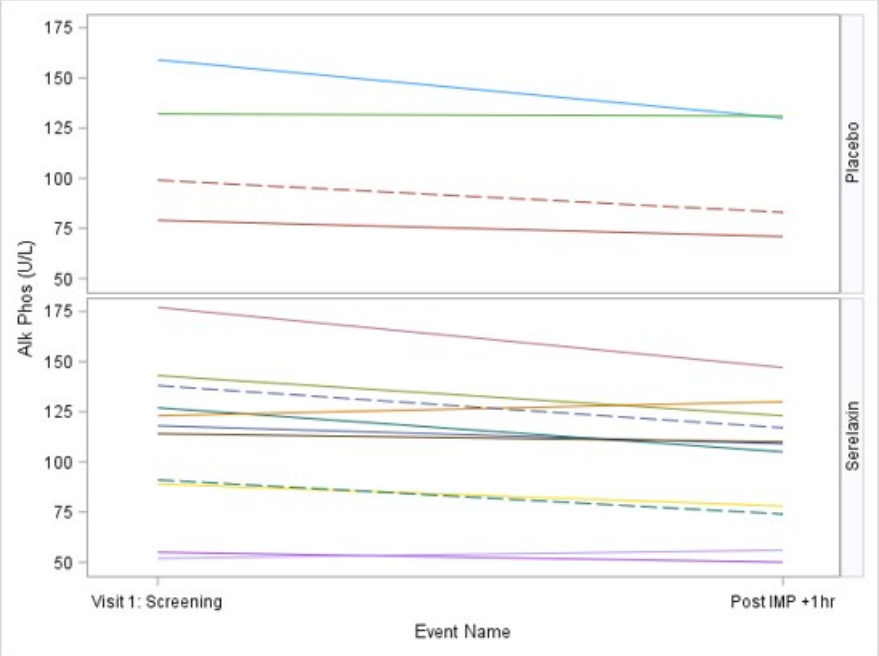
Serum aspartate aminotransferase



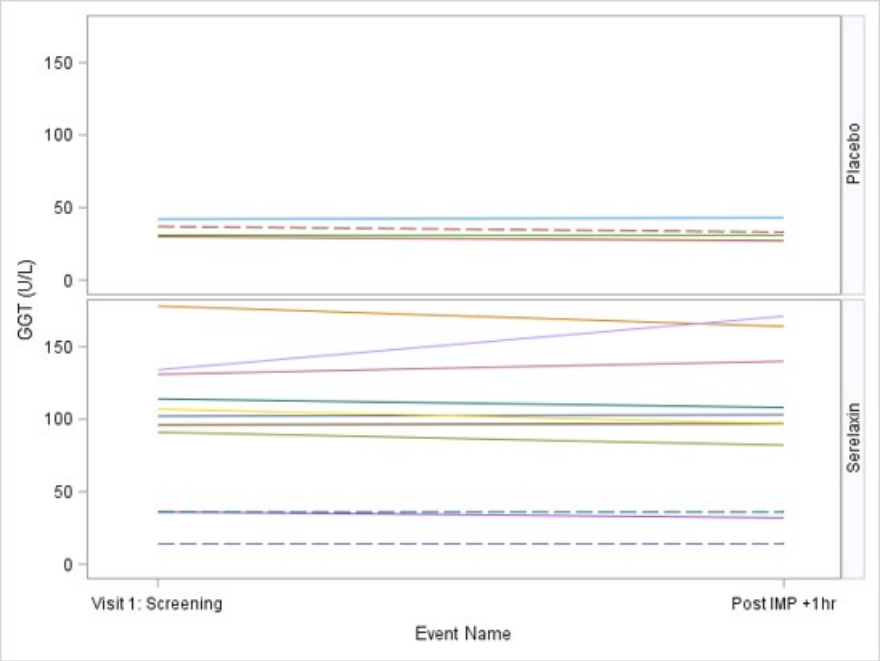
Serum alanine aminotransferase



Serum alkaline phosphatase



Serum gamma glutamyl transpeptidase



13b. Adverse events

Treatment with serelaxin was well-tolerated. Overall, 12 adverse events (AEs) were reported in 7 subjects treated with serelaxin. None were serious or considered to be related to the IMP. The following table shows the breakdown of AEs by treatment arm.

	Treatment				All	
	Placebo		Serelaxin			
	N	%	N	%	N	%
Total	4	100	11	100	15	100
At least one recorded AE						
No	3	75	4	36	7	47
Yes	1	25	7	64	8	53

The table below gives details of the AEs recorded. There were no SAEs in this study. There were no pregnancies reported.

- **Placebo Arm:**

Study ID	Adverse Event	Start date	Is this a SAE/SAR?	Severity	Related to IMP	Related to NIMP	Related to interaction IMP-NIMP	Expectedness	Outcome	Date resolved
002	Diarrhoea - dark green	08NOV2017	No	Mild	Possibly related	Possibly related	Unrelated	Unexpected	Resolved	08NOV2017

- **Serelaxin Arm:**

Study ID	Adverse Event	Start date	Is this a SAE/SAR?	Severity	Related to IMP	Related to NIMP	Related to interaction IMP-NIMP	Expectedness	Outcome	Date resolved
003	Syncopal episode on inserting venflons	29NOV2017	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Resolved	29NOV2017
003	Syncopal episode on inserting hepatic catheter	29NOV2017	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Resolved	29NOV2017
003	Syncopal episode on removing hepatic venous catheter	29NOV2017	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Resolved	29NOV2017
003	Reports mild ache in right upper quadrant at 24 hour phonecall	30NOV2017	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Ongoing	.
005	Mean diastolic BP 58.3mmHg at IMP + 30mins	20DEC2017	No	Mild	Possibly related	Unrelated	Unrelated	Expected	Resolved	20DEC2017
006	Prolonged QTin ECG at IMP + 2 hours	17JAN2018	No	Mild	Possibly related	Unrelated	Unrelated	Unexpected	Resolved	17JAN2018
008	Bilirubin rise	07FEB2018	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Resolved	12FEB2018
012	Prolonged QTc on ECG	30APR2018	No	Mild	Unrelated	Possibly related	Unrelated	Unexpected	Resolved	30APR2018
014	Syncopal episode whilst inserting venflon	13JUN2018	No	Mild	Unrelated	Unrelated	Unrelated	N/A	Resolved	13JUN2018
014	Syncopal episode whilst positioning hepatic catheter	13JUN2018	No	Mild	Unrelated	Unrelated	Unrelated	N/A	Resolved	13JUN2018
014	Dental Abscess	27JUN2018	No	Mild	Unrelated	Unrelated	Unrelated	Unexpected	Resolved	04JUL2018
016	Right femoral arterial puncture	18JUL2018	No	Mild	Unrelated	Unrelated	Unrelated	N/A	Resolved	18JUL2018

14. Discussion

14a. Limitations

The study was terminated before the recruitment target was met due to a worldwide lack of serelaxin (Novartis have stopped manufacturing serelaxin and there was none available with a shelf-life beyond 31st August 2018). Therefore, based on our sample size calculation, the study is underpowered to detect the primary endpoint.

The study was double-blind, placebo-controlled which would have addressed potential sources of bias.

There was one **protocol violation**:

Violation No#	Participant No#	Date Occured	Description	Action
1	005	20DEC2017	On two occasions during the 2 hour IMP infusion the participant's diastolic BP fell below 60 mmHg. These results are fractionally below the lower limit set in the protocol. It was not recognised at the time in error, in part as the investigators and nursing staff do not consider a DBP of 58 or 59 in a well participant to be clinically significant. The systolic BP was well above the systolic cut off of 90mmHg (127-145mmHg). The participants vital signs were being closely observed with both continuous MindRay monitoring and regular observations. NEWS reamined at 0 and the participant was completely asymptomatic, remaining well throughout.	CA - The study drug is now complete therefore no corrective action can be taken. Participant followed up following day. ACCORD made aware and violation reporting form completed. PA - The stopping criteria will be printed and made available to staff during infusion days as a poster. The details of this event have been disseminated to the team as a reminder.

All **protocol deviations** captured in the sponsor`s database are presented in the table below:

Participant No#	Date Occured	Description	Corrective and preventative actions
001 - 010 (inclusive)	20MAR2018	Protocol states that total protein will be checked on bloods however this has not been performed. Picked up at monitoring. Not noticed as not clinically relevant.	CA - deviation noted. PA - Craig Marshall emailed and we have requested to add total protein to order set
001	25OCT2017	Screened at 1hr, the waveform had changed. Fluoroscopy confirmed line tip in inferior vena caua. Unable to finance line without interventional radiologist therefore unable to obtain 1hr HVPG.	CA - Contacted interventional radiologist for support. PA - Consider radiologist support at HVPG timepoints. Consider use of additional guideline in future. Encourage participants not to move
001	25OCT2017	ICG infusion ran out prior to line re-positioning in hepatic vein at time of 2nd ice sampling. Samples obtained although imaging suggested line tip in inferior vena cava. 26OCT17 - Above deviation confirmed on analysis of ICG results	CA - attempted to re-position prior ot end of ICG infusion. PA - Plan to start ICG bolus and latek in order to ensure sufficient time for ensure accurate line position
001	25OCT2017	POST IMP + 30 mins. BP and heart rate only taken once in error. Not taken in duplicate. Nurse measuring observations was unaware.	CA - All study staff reminded about requirement to take HR and BP in triplicate. PA - Should not happen again now that all nursing staff aware. Remind staff at each study visit
001	25OCT2017	Respiratory Rate not recorded at observations post imp + 30 mins. Nurse taking observations at this time point forgot it was needed.	CA - All staff reminded of the time points at which respiratory rate is required. PA - Remind staff at each study visit. Refer to paper CRF document protocol at each stage.
001	25OCT2017	Due to a delay in the planned dose time the 1 hour pre-IMP infusion measurements were performed outwith the 1 hour window recorded in the protocol. This will not alter the validity of the results but is a deviation from the protocol.	CA - Attempted to get line in position and start IMP as quickly as possible to minimise delay. PA - In future: use a guidewire +/- have interventional radiologist present to ensure efficient line positioning
001	25OCT2017	Protocol states that 2% of lidocaine should be used. On this occasion 3% lidocaine was used. Apprpriate LA was achieved but this was a deviation from protocol	CA - Deviation log completed. Nursing/medical staff reminded that protoocol stiulates that 2% should be used. PA - Always check that 2% lidocaine is selected for line insertion. ONly 2% lidocaine will be removed from cupboard.
001	25OCT2017	Protocol states that the following tests are performed at IMP + 2 hours (+/- 15 mins) HVPG measurement. ICG and biomarker bloods. Bio-impedence (cardioscreen). IVPC measurements. APWN (tensioned). Obs. ECG in duplicate. Due to difficult with line position we were unable to perform all of the above tests within the 30 minute window	CA - Effords made to position the line as swiftly as possible. PA - Start these measurements 15 mins before IMP and 2 hours timepoint. We must try to obtain results swiftly in order to perform everything within 30 minutes. The priority is the primary endpoint of HVPG at 2 hours. If this is a continual deviation then consider amendment to wider window to IMP + 2 hours +/- 20
001	25OCT2017	Post IMP + 1hr respiratory rate not recorded - staff error	CA - All staff reminded of the time points at which HR, RR + BP required. PA - Ensure protocol and pCRF are consulted at time of obs
001	25OCT2017	IMP + 30 min overdose - reading taken (16.7ml) taken at 12.05. This was at IMP + 37 minutes. 7 minutes late. Able to calculate back and confidently confirm no overdose (13.5ml in 30 mins)	CA - Documented as deviation. PA - Appoint someone to watch clock specifically for pump checked at appointed times
001	25OCT2017	ECG at post IMP + 2 hours. ECG = 16455. ECG2 = 1648, ECG3 = 1650. Gap between ECG 1 and 2 = 3 mins. not 2 mins	CA - Documented as deviation. PA - Encourage patient and staff to stand still at time of ECG so as not to have any interference and a time error
001	25OCT2017	Post-IMP +2.5 hour vital signs taken at 1700, 1703, 1704. 3 minute gap between V5 1+2 protocol states 1-2 min gap.	CA - Documented as deviation. PA - Minimise distractions. Watch the clock. Try to record BP nearer 1 min allowing leeway
001	25OCT2017	Post-IMP + 3.5 hour vital signs 1800, 1804, 1806. V5 1+2 = 4min apart	CA - Documented as deviation. PA - Minimise distractions. Watch the clock. Ensure all staff aware of the importance of time gaps.

002	08NOV2017	WHVP at IMP + 3. Both FHVP and WHVP recorded in triplicate as per protoocol. 3rd WHVP (recoreded in pCRF as 24 and seen by all study staff on day) did not capture on mindray machine and no paper same data available. Number does not alter mean WHVP and therefore does not have significant afffects on results	CA - Try to be sure that all pressures have been captured. PA - Capture each results in duplicate to try to prevent this happening
002	08NOV2017	Tensioned arteriogrpah recording of APWV was completed just outwith the appointed window at IMP + 2 hours +/- 15 mins	CA - Tried to be as fast as possible performing all necessary tests at this timepoint. PA All study staff aare of need to be efficient in this short window. Start these tests at 15 mins before IMP + 2 hours + perform them as efficiently as possible
002	08NOV2017	Screening ECG 11.43 1145 1148 = 3 mins apart	CA - Documented as deviation. PA - minimise movement. Switch off electric bed and other electrical items nearby to minimise electrical background interference.
002	08NOV2017	3.5ml lidocaine given where protocol states 10ml	Sufficient anaestheticgiven for patient fiven bodysize. 10 ml will not be needed in full for every patient. Would not give more than is required to achieve adequate anaesthetic
002	08NOV2017	IMP + 30 min overdose. Pump checked 2 mins late ie at 32 mins. Able to calculate dose at 30 mins and confidentally confirm no overdose.	Appoint a nurse to ensure pump checked at correct time points
002	08NOV2017	IMP+30 mins vital signs taken 10 minutes early therefore 40 minutes gap betweem this and the next vital signs	Unsure why this may have occured. Ensure all staff know to stick to exact time points where possible
002	08NOV2017	Post-IMP + 2W ECG. ECG1 - 1544. ECG2 - 1546. ECG3 - 1549	Try to minimise interference to be able to achieve adequae ECG tracing at the night times switch off phones and stand still
003	22NOV2017	Gap between ECG 2 + ECG 3 was 2 mins and 20 seconds rather than 2 mins	CA - Ask the patient not to move or talk during ECGs. PA - Continue to try to time the ECGs accurately ensuring the patient doesnt move or talk at the point of recording where possible
003	29NOV2017	IVCOP not recorded pre-IMP. Line positioned rapidly in heptatic vein and IVCP not recorded. Team decision not to move line when positioned so well in liver	CA = Ensure IVCP is measured post-IMP PA - Screen line in slowly and try to ensure that the IVGP is not missed. highight IVGP on reminders of study plan
003	29NOV2017	Participant uncomfortable after 2 hours of IMP and keen to complete proceedings. Fidgety on trolley and therefore despite two attempts no data was collected APWV. Decision made not to try again for patient comfort.	Continue to prioritise patient comfort. Encourage participant that the most intense part if the study is almost over and that the data is important.
003	29NOV2017	Volume of IMP infused not checked at IMP + 2 hours timepoint. Checked at time of infusion stopping (2 hours and 33 mins) and no evidence of an overdose	CA - documented on pCRF that volume infused at 14.09 was not a 2 hour recording. PA - Ensure that the pump is checked at exactly 2 hours. Allocate a nurse to priorisatation tasks
003	29NOV2017	post-IMP + 2 hours ECG 2 - 3 gap = 1 min and 13 seconds rather than 2 min	CA - ensure no distractions during time of taking ECGs. PA - minimise distractions. Ask patient not to talk. Watch clock to ensure 2 min gap
003	29NOV2017	Final vital signs at post-IMP + 4 hours taken 10 mins early. Patietn unwilling to wait longer. Patient was monitored in the CRF until 4 hours post-IMP however	CA - Times documented on pCRF and devation recorded. PA - Try to be as swift as is safely possible during study period to ensure IMP and 4 hours is not too late into the evening. Encourage page thay the timings of the vital signs are important for dat collection.
003	22NOV2017	Pump check (IMP infusion) & IMP+30 mins timepoint was 2 minutes late. No overdose had occured	Appoint someone to watch clock at specific timepoint and prompt checking of infusion
003	22NOV2017	6ml lidocaine given where protocol states 10 ml to be given.	Sufficient local anaesthetic given fo patient given body habitus. 10ml will not be needed in full for each patient. Would not give more than required to achieve adequate anaesthesia
004	30NOV2017	ECG performed 1 min 59 secs after ECG 1. The gap between ECGs was 1 second too short	CA - Recognise error and try to match times more accurately. PA - Try to minimise distractions dureing ECG period. Give just over 2 mins to limit risk of similar.
004	06DEC2017	Start of IMP + 2 hour pump check performed 21 minutes later due to busyness of 2 hour time window	CA - PUmp volume recorded at 2 hours 21 minutes when error recognised. PA - Verbal reminder at 2 hours to check and record pump volume
004	06DEC2017	5ml lidocaine given where protocol states 10 ml to be given.	Sufficient local anaesthetic given fo patient given body habitus. 10ml will not be needed in full for each patient. Would not give more than required to achieve adequate anaesthesia

005	20DEC2017	APWV result difficult to acquire ? fault with machine or patients vessels difficult to assess. Pre-IMP APWV reading recorded on 4th attempt. NO duplication reading taken. IMP + 2 hour APWV reading achieved on 3rd attempt and not duplicated	CA - attempts made to re-positions and tighten cuff. Parameters chagend slightly on machine to acquire reading. Patient too uncomfortable to repeat. PA - Try to be accurate as possible wuth jug-symph measurement, tighten cuff and repeat test if possible to get 2 good readings
005	20DEC2017	Complicated kine/catheter insertion due to anatomy of heptatic vein. ICG infusion commenced at appropriate time however whole infusion finshed before line in hepatic vein/ No samples obtained with no baseline ICG results. and no infusion left, ICG samples not taken at IMP + 2 hours.	CA - Allowed ICG for pre-IMP + volume assigned for IMP + 2 hour period to run in the pre-IMP period ie 2 x normal volume with plan to make up another batch if required. PA - Start ICG as late as possible without causing undue delay to the study visit. UNpreventable deviaton
005	20DEC2017	Given technical problems with line placement, catheterused which is wedged by position rather than balloon. For this reason, obtaining HVPG at 1 hour of IMP considered too labour intensive for participant and interventional radiologist - not obtained	CA - Documented decision in pCRF. Decision made to protect patient and only record HVPG when necessary and interventional radiologist support available. PA - Unavoidavble due to practical difficulties and interventional radiology support.
005	20DEC2017	No IVCP measurement recorded in IMP + 2 hour period	CA - Line removed before IVCP could be recorded. Patient uncomfortable and efforts made to finish quickly. PA - Verbal and visual reminder tp record IVCP before line removal
005	20DEC2017	Volume informed check @ IMP + 30 min timepoint was 3 minutes late. No overdose had occured	Make sure someone is checking clock at specific timepoint and prompting the checking of infusion
006	17JAN2018	ECG2 at timepoint 'PRE-IMP ECG was un-interpretable. Poor training with variation in baseline and last 2 inches of ECG has not printer	CA - ECG 1 + 3 reviewed and caused no concern. PA - Ensure all team members aware of need to thoroughly check ECG when printed. Switch off unnecessary electrical equipment which may cause interference and all team members standing still whilst ECG acquired as machine is very sensitive.
006	17JAN2018	ECG 3 of pre-IMP (infusion ok) timepoint was poor trace. PI unable to interpret	CA - Documented as deviation. ECG1 and ECG2 more interpretable. PA - Ensure patient and staff still at time of ECG recording. Favour good ECG trace over keeping to 2 minute window.
006	17JAN2018	ICG (2) infusion ran for 38 minutes where protocol states it shuld run for at least 40 mins	CA - Samples taken 2 mins apart ie 38 mins, 40 mins, 42 mins to confirm equilibrium reached. PA - Delay start of second ICG infusion if volume of ICG infusion if volume of ICG left is limited so that no variation in start time is necessary.
007	24JAN2018	ECG@Pre-IMP (infusion ok). ECG1 11.21(55); ECG 2: 11.24(06); ECG3: 11.26. Interval between ECG1 and ECG2 3 minutes rather than 2 minutes (actually 5 seconds outwith)	Watch clocks closely for minutes and records between ECG recordings to ensure 2 minute gap.
008	07FEB2018	HUPG reading at IMP + 1 hour. Catheter no longer in correct position at 1 hour. Insufficient time to re-position under fluoroscopic guidance. IMP + 1 hour HUPG not measured	CA - Plan to get radiographers and interventional radioligist round in good time for IMP + 2 hour HUPG which is the primary outcome. PA - Unavoidable. Try to encourage patient tolie still however position of catheter is influence by breathing
009	28FEB2018	Unable to position balloon-tipped catheter in the hepatic vein, despite help from the interventional radiologist. Decision made to abandon procedure due to anatomical/procedural complations. IMP not started.	Much time spent trying to gain access to hepatic vein. Unavoidable.
009	28FEB2018	Blood pressure readings at post-IMP + 2 hours. 3 minute gap between blood pressure readings	CA - Unable to rectify at time. Recorded here as deviation. PA - Minimise distractions. DO not talk and ensure people recording blood pressure are aware of 1-2 min gap
009	28FEB2018	Blood pressure readings at post-IMP + 3 hours. 1 minute gap between blood pressure readings 2 and 3	CA - Nurse involved asked participant if she could take another BP reading to allow 1-2 min gap but participant not keen due to arm discomfort. PA - Ensure nurse focuses on clock when taking BP readings

009	28FEB2018	Red weather warning acorss Edinburgh. Government warning not to drive after 3pm. Decision made to not keep patient until post IMP + 4h ours for further obs. Patient not given IMP anyway. No evidence of bleeding from site. Risk to patient greater if discharge delayed. Allowed home	CA - Confident that best decision made for the participant. PA - Consider not running a study day at all if severe weather warning
010	14MAR2018	Unable to cannulate the hepatic vein with balloon-tipped catheter despite multiple attempts. Procedure abadoned.	CA - Multiple attempts and different doctors tried to position catheter with no success. PA - Decision to obtain a different type of balloon-typed catheter so to have another alternative catheter which is less rigid and should help with difficult patients
010	14MAR2018	Post-IMP + 2 hour ECGs. ECG1 to ECG2 & ECG2 to ECG3 all 3 minutes apart. New nurse involved in study.	CA - noted as deviation. PA - Re-emphasised. Ensure nuyrsing staff reading pCRF to reminded themselves of time gaps during day.
010	14MAR2018	PostIMP + 2 hour observations (HR+BP) obs 2 and 3 were 3 minutes apart (protocol states 1-2 mins). New nurse involved in study.	CA - noted as deviation. PA - Re-emphasised. Ensure nuyrsing staff reading pCRF to reminded themselves of time gaps during day.
012	30APR2018	Vital signs at IMP +1hr were recorded 7 mins late as staff involved in other study activities.	CA - Vital signs recorded at earliest possible moment. PA - Try to appoint a member of the team to ensure vital signs are taken at necessary points
012	30APR2018	Team unable to obtain HVPG at IMP+ 1hr timepoint as hepatic catheter had moved during first hour of IMP	Radiographers called early to attend to ensure catheter repositioned for IMP+2hour (primary endpoint)
012	30APR2018	IMP volume infused was meant to be recorded at IMP +1.5 hour but was recorded at 1hr 35 minutes instead. 5 mins late	Try to appoint a nurse specifically for checking pump volume at desigated points
014	13JUN2018	Unable to obtain HVPG at 1 hour. Difficult HVPG at 0 hour although readings obtained. No fluoroscopy available at 1 hour - allow repositioning therefore HVPG not obtained.	CA - Radiographers called early to ensure 2hr HVPG which is primary outcome. PA - Unpreventable but continue to try to position well at HVPG at 0 hour
014	13JUN2018	3x serum ICG blood samples not processed correctly. Remained out of fridge for >24 hours. Samples not usable	CA - Processed as soon as discovered however subsequently they informed by PI samples not usable. PA - Staff education, highlighted regime.

14b. Generalisability

To demonstrate generalisability, these initial trial findings would need to be externally validated in a larger more diverse study population, ideally with patients stratified by baseline HVPG into mild (5-10 mmHg) and clinically-significant (>10 mmHg) portal hypertension.

14c. Interpretation

The aim of the STOPP study was to investigate the safety and efficacy of the vasoactive peptide molecule serelaxin (recombinant human-2 relaxin) in reducing portal pressure, as determined by the HVPG in patients with compensated cirrhosis and clinically-significant portal hypertension. It is important to note that the trial was terminated before the recruitment target was met; consequently, although serelaxin had a neutral effect on HVPG in the treated sample, low statistical power increases the probability of a type II error.

Portal hypertension is the strongest predictor of decompensation and death in patients with compensated cirrhosis (Ripoll C et al, Gastroenterology 2007) and the major driver for serious complications such as variceal bleeding, ascites and hepatic encephalopathy. At present, non-selective beta-blockers, vasopressin analogues and somatostatin analogues are the mainstay of drug treatment for portal hypertension, but these strategies are suboptimal and only target splanchnic hyperaemia. New therapeutic options, particularly drugs that reduce increased intrahepatic vascular resistance in cirrhosis are needed. In preclinical models, serelaxin decreased portal pressure through an increase in intrahepatic nitric oxide signalling and a reduction in hepatic stellate cell contractility (Fallowfield JA et al, Hepatology 2014). In an initial small exploratory open-label phase II study, serelaxin induced a rapid and

potentially clinically significant reduction in portal pressure in patients with cirrhosis, portal hypertension and a transjugular intrahepatic portosystemic shunt (TIPSS) (Lachlan NJ et al, Hepatology 2015).

A consistent finding in this (and previous) studies is the good safety profile of serelaxin in patients with cirrhosis and portal hypertension. With 2 hr of serelaxin infusion, there were no newly occurring liver enzyme abnormalities, no clinically significant changes in blood pressure, and no discontinuations due to AEs. Additionally, in a separate study the pharmacokinetic and safety profiles of serelaxin were not affected in patients with mild, moderate or severe hepatic impairment (Kobalava Z et al, Br J Clin Pharmacol 2015). In contrast, terlipressin is associated with a high risk of serious (particularly ischaemic) complications (Gifford FJ et al, Aliment Pharmacol Ther 2017).

Mechanisms of portal hypertension differ in patients with mild portal hypertension (HVPG >5 but <10 mmHg) compared to those with clinically-significant portal hypertension (HVPG > 10 mmHg) (Bosch J et al, J Hepatol 2015). In mild portal hypertension the main mechanism leading to raised portal pressure is increased intrahepatic vascular resistance, while in those with clinically-significant portal hypertension/varices, increased portal flow plays a major role in maintaining and aggravating the portal hypertensive state. These pathophysiological differences can influence drug efficacy depending on the stage of disease and the predominant mechanism of action. For example, patients with mild portal hypertension have a significantly lower response to non-selective beta-blockers (which decrease portal flow) compared to those with clinically-significant portal hypertension/varices, who have a hyperkinetic circulation (Villanueva C et al, Hepatology 2016).

In this study, serelaxin had a neutral effect on HVPG and a range of secondary haemodynamic endpoints in a population of patients with HVPG > 10mmHg. It is possible, given the proposed mechanism of action of serelaxin in cirrhosis (decreased intrahepatic vascular resistance), that it may have a more pronounced effect on portal pressure in patients with mild portal hypertension. We recruited patients with HVPG > 10 mmHg because these individuals are at most risk of decompensation and a decrease in portal pressure in this population would potentially lead to a reduction in clinically-meaningful endpoints (e.g. development of varices, variceal bleeding and ascites).

The acute haemodynamic effects of vasoactive drugs (e.g. propranolol, nadolol, vasopressin, terlipressin, somatostatin) on portal pressure have generally been demonstrated 15-20 minutes after intravenous administration (Villanueva C et al, Gastroenterology 2009; Baik SK et al, Am J Gastroenterol 2005). Here, serelaxin was administered over a relatively short time-frame (2 hr), at least in part because rapid changes in visceral blood flow had been observed in a previous Novartis-sponsored study in a similar population (X2201). However, for drugs acting on intrahepatic vascular resistance, previous studies have been much longer (e.g. simvastatin significantly decreased HVPG after 28 days of oral administration (Abraldes JA et al, Gastroenterology 2009)). So, it is conceivable that potential changes in HVPG due to a reduction in intrahepatic vascular resistance and/or anti-fibrotic/anti-inflammatory mechanisms were not captured after only a short serelaxin infusion. Whether any portal pressure reducing effect of serelaxin might be demonstrated with a longer administration would need to be verified in a longer, adequately designed study, if formulation or half-life issues can be resolved to enable chronic exposure to serelaxin (or other RXFP-1 agonist).

Finally, we have never performed a dose-ranging study of serelaxin in cirrhosis. We used the same infusion regimen that had shown encouraging haemodynamic effects in our previous exploratory study and achieved similar steady-state serum concentrations to that observed in our 72 hr rat cirrhosis models (Fallowfield JA et al, Hepatology 2014) and in human heart failure following 48 hr i.v. infusion

(Teerlink JR et al, Lancet 2013). However, biological effects of relaxin are known to follow a U-shaped dose-response curve (Danielson LA, J Appl Physiol 2003) and we do not know if serelaxin might have induced more pronounced effects on HVPG and the secondary haemodynamic endpoints at higher (or lower) doses. Future work should address dose-response relationships.

14d. Conclusion

In summary, this exploratory randomised study showed that an i.v. infusion of serelaxin for 2 hr was safe but had a neutral effect on portal pressure in patients with cirrhosis and clinically-significant portal hypertension (HVPG > 10 mmHg). Future studies might evaluate the acute effect of serelaxin on *mild* portal hypertension (HVPG 5-10 mmHg) and/or the effect of *chronic* administration of serelaxin on hepatic fibrosis and portal pressure.

15. Other Information

15a. Registration

The study was registered at ClinicalTrials.gov (Identifier: NCT02669875), February 1st 2016.

15b. Protocol

The full study protocol (Version 5.0, 11Jun2018) is available on request from the Principal Investigator.

15c. Funding

The study was funded as an Investigator Initiated Trial (IIT) by Novartis Pharmaceuticals UK Ltd. The funders reviewed, requested revisions pertaining to, and approved the study Protocol. The funders had no role in the conduct of the research or the subsequent analysis.

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