NOVARTIS

Clinical Development

RLX030A/Serelaxin

CRLX030A2302 / NCT02007720

A multicenter, randomized, double-blind, placebo controlled phase III study to evaluate the efficacy, safety and tolerability of serelaxin when added to standard therapy in acute heart failure patients

RAP Module 3 – Detailed Statistical Methodology

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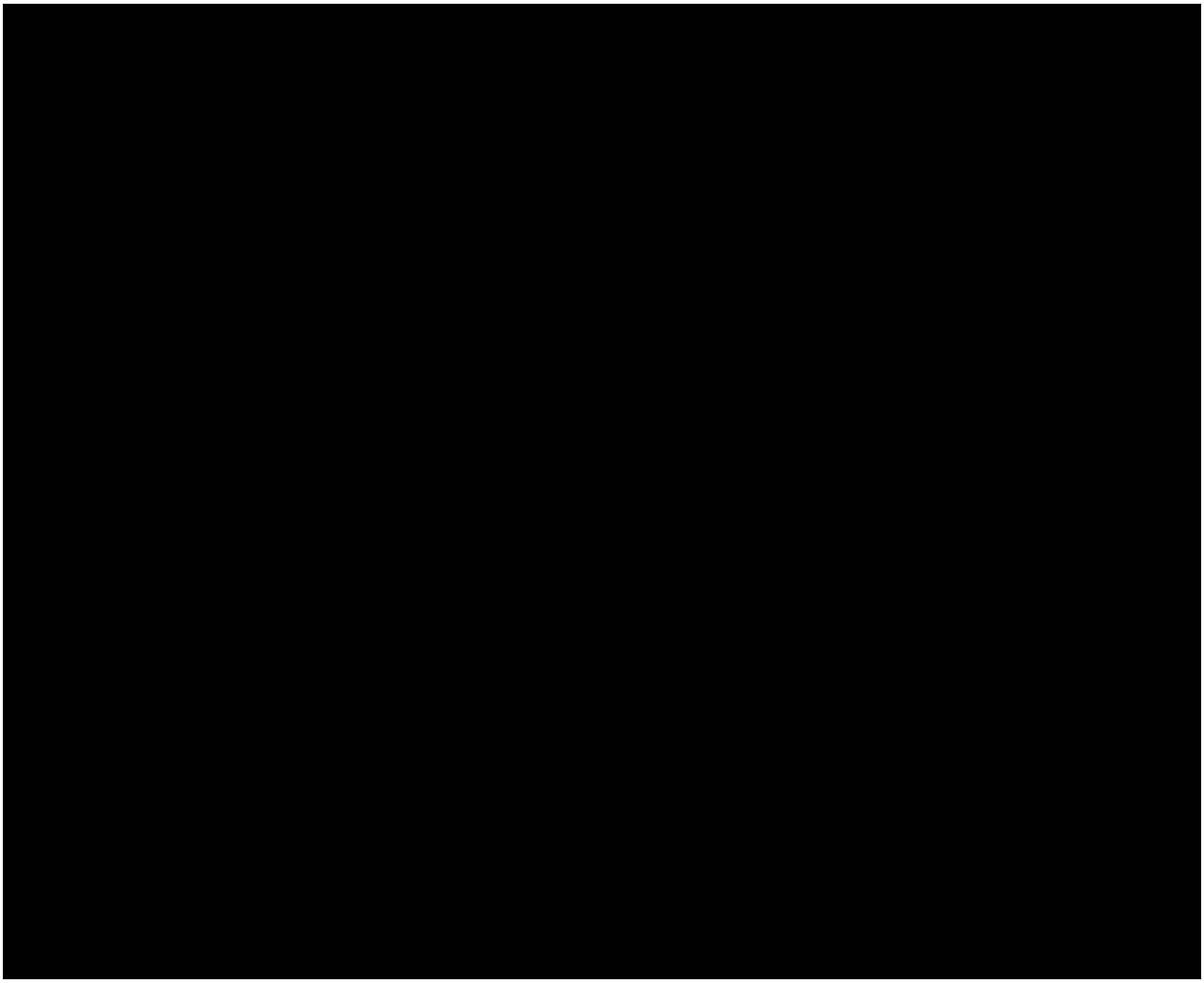

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ACE	Angiotensin Converting Enzyme
ACM	All-cause mortality
AE	Adverse Event
AHF	Acute Heart Failure
ALP	Alanine Phosphatase
ALT	Alanine Aminotransferase
ARB	Angiotensin-II Receptor Blocker
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
BLQ	Below limit of quantification
BNP	Brain Natriuretic Peptide
BP	Blood pressure
BPDE	Blood pressure decrease event
BUN	Blood Urea Nitrogen
CBPDE	Confirmed blood pressure decrease event
CCU	Coronary care unit
CEC	Clinical Endpoints Committee
CI	Confidence interval
CL	Clearance
CPK	Creatinine Phosphokinase
CRF	Case Report Form
CSR	Clinical Study Report
Css	Steady state concentration
[redacted]	[redacted]
CV	Cardiovascular
CVD	CV death
CVDR	CV death or rehospitalizaiton due to heart failure or renal failure
CWHF	Clinical worsening heart failure
dL	Deciliter
DMC	Data Monitoring Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EF	Ejection fraction
eGFR	Estimated Glomerular Filtration Rate
FAS	Full Analysis Set
HF	Heart failure
ICU	Intensive Care Unit
ITT	Intent-to-treat
IV	Intravenous
kg	Kilogram

LFT	Liver Function Toxicity
LLOQ	Lower limit of quantification
LOS	Length of hospital stay
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
min	Minute
mL	Milliliter
mmHg	Millimeters mercury
NYHA	New York Heart Association
PASS	Physician assessment (of HF) signs and symptoms
PD	Protocol Deviation
PPS	Per Protocol Set
PT	Preferred Term
RAN	Randomized Set
RAP	Report and Analysis Preparation
RBC	Red Blood Cell
SAE	Serious adverse event
SAF	Safety Set
SBP	Systolic Blood Pressure
[redacted]	[redacted]
SGOT	Glutamic Oxaloacetic Transaminase
SGPT	Glutamic Pyruvic Transaminase
sMDRD	Simplified Modification of Diet in Renal Disease
SMQ	Standardized MedDRA Query
SOC	System Organ Class
TBL	Total Bilirubin
ULN	Upper Limit of Normal
VAS	Visual analog scale
WHF	Worsening heart failure
WHO-DD	World Health Organization Drug Dictionary
µg	Microgram

1 Introduction

The purpose of this document is to outline the planned analyses to be completed to support the completion of the Clinical Study Report (CSR) for protocol CRLX030A2302 (also referred as Study RELAX-Asia).

This study is to evaluate the efficacy, safety and tolerability of intravenous infusion of 30 µg/kg/day serelaxin for 48 hours, when added to standard therapy, in approximately 1,520 Asian acute heart failure (AHF) patients.

2 Statistical methods planned in the protocol and determination of sample size

[redacted]

Data will be analyzed according to the data analysis section 9 of the study protocol which will be included in Appendix 16.1.1 of the CSR.

Unless otherwise stated, summary tables/figures/listings will be on all subjects included in the population under consideration. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, minimum, and maximum will be presented. Kaplan-Meier curves will be provided for time-to-event variables.

[redacted]

2.1 Subject and treatment

2.1.1 Analysis population

For analysis purposes, subjects will be classified accordingly into following analysis sets as defined below:

- **Screened set (SCR)** – All subjects who signed the informed consent. The SCR includes only *unique screened subjects*.
- **Randomized population (RAN)**: All subjects who received a randomization number, regardless of receiving trial medication
- **Full analysis set (FAS)**: All subjects in the RAN population who were not misrandomized subjects*. Following the intent-to-treat (ITT) principle, subjects are analyzed according to the treatment they have been assigned to at the randomization.

  Misrandomized Subjects are those who have not been qualified for randomization, have been inadvertently randomized into the study and who did not take study drug. Misrandomized subjects are defined as cases where IRT/IWRS contacts were made by the

site either prematurely or inappropriately prior to confirmation of the patient's final randomization eligibility and double-blind medication was not administered to the patient. These subjects should subsequently be discontinued from the study.

- **Safety population (SAF)**: All subjects who received any amount of study drug and have at least one post-baseline safety assessment. Of note, the statement that a patient had no adverse events also constitutes a safety assessment. Subjects will be analyzed according to treatment received. Subjects who received any amount of serelaxin will be included in the serelaxin treatment group.
- **Per-protocol population (PPS)**: All subjects in the FAS who received any amount of study medication and who do not have any protocol deviations that could confound the interpretation of analyses conducted on the FAS. This PPS is used to assess robustness of the primary and key secondary analysis results.

Note subjects who did not meet inclusion/exclusion criteria but inadvertently randomized and received the study drug are not referred as misrandomized subjects per definition in this trial. They will be included in RAN, FAS, SAF populations but not in PPS.

2.1.2 Protocol deviation (PD) and subject classification

In general protocol deviations will be categorized as following:

- Selection criteria not met
- Subject not withdrawn as per protocol (*not defined in the study*)
- Treatment deviation
- Prohibited concomitant medication (*not defined in the study*)
- Other

Protocol deviations are identified prior to clinical database lock/unblinding and entered into a dedicated data panel as part of the locked database. [redacted]

The major protocol deviations resulting in exclusion of subjects from an analysis population are outlined in the table below:

Table 2-1 Major protocol deviations resulting in exclusion of subjects from analysis populations

		Excluding from analysis set			
PD category	**Deviation**	**RAN**	**FAS**	**SAF**	**PPS**
Selection criteria not met	Missing informed consent (INCL01)		x	x	x
	Patient hospitalized for reasons of non-AHF event (INCL04)				x
	Systolic BP <125 mmHg at the start and/or at the end of screening (INCL08)				x
	Time from presentation to randomization >16 hours (INCL09)				x
	Dose of IV furosemide between presentation and				x

		Excluding from analysis set			
PD category	**Deviation**	**RAN**	**FAS**	**SAF**	**PPS**
	start of screening is < 40 mg (INCL10)				
	eGFR is <25 or > 75 mL/min/1.73m2 at screening (INCL11)				x
	Dyspnea is primarily due to non-cardiac causes (EXCL01)				x
	Sepsis or active infection requiring IV anti-microbial treatment reported at screening (EXCL26)				x
	AHF is due to significant arrhythmias (EXCL04)				x
Treatment derivation	Serelaxin infusion administered in dilutent other than the recommended 5% dextrose (TRT02)				x
	Wrong study drug kit administered (TRT06)				x
	Subject misrandomized (TRT11)		x	x	x

All exceptional cases, problems and the final decisions on the allocation of subjects to populations will be fully defined and documented before data base lock (in particular before breaking the blind where applicable) and will be fully identified and summarized in the clinical study report.

Table 2-2 Non-protocol deviation criteria resulting in exclusion of subjects from analysis populations

	Excluding from analysis set			
Criterion	**RAN**	**FAS**	**SAF**	**PPS**
Subject randomized but not receiving any dose of double-blind study medication			x	x

2.1.3 Time window, baseline and post-baseline definitions, and missing data handling

Screening phase (Hour -16): The screening phase is the time period prior to randomization, which should be within 16 hours after presentation. After obtaining written informed consent, the subject will be evaluated for eligibility to participate in to the study.

All references to index hospitalization refer to the initial hospitalization for acute heart failure (AHF).

Baseline (Hour 0/Day 1): The assessments identified in the eCRF as baseline assessments will be used as the baseline reference for all analyses. The baseline date and time will be considered the date and time of initiation of study drug, or randomization if the subject was not treated with study drug. If the value is missing, the last value at screening visit will be used.

Post-baseline phase: The post-baseline phase begins at the time when study drug administration starts (Hour 0/Day 1) and ends with the last study assessment/last follow-up or the death of subject. For subjects who did not receive study drug, the post-baseline phase begins at time of randomization.

Time window: For the scheduled post-baseline visits, time window is not defined; the data will be used as reported in eCRFs (i.e., the actual assessment times will not be used to

reclassify the time point at which a measure was taken). The by-visit report will be presented by protocol scheduled visit. Time-to-event analyses will be based on actual date and time as reported when available.

Missing data handling

Missing data handling for efficacy endpoints are discussed in Section 2.2, Efficacy evaluation. In general, missing values of other assessment/measurement will not be imputed unless otherwise specified.

The dates for efficacy endpoint events and AE events should be reported as instructed in eCRFs. If a partially missing date cannot be resolved after data query, the following is the general rule for imputation as necessary only if the calculation is needed (e.g., to calculate time-to-event):

a) if only the month of the event is known, then the 15th day of this month will be imputed for a missing day in case the month and year are after the month and year of double blind treatment start. If the month and year are equal to the double blind treatment start then the event date will be imputed by the treatment start date.
b) if only the year of the event is known, then the 1st July will be imputed for a missing day and month in case the 1st July is after double-blind treatment start (otherwise treatment start date will be used), or
c) for events occurring between two visits after randomization, without other information available, the date/time in the middle of these visits will be used.

[redacted]

Adverse Events with incomplete start dates will be considered treatment emergent (incident) if it is not obvious from the partial date whether the event onset was after study drug was initiated.

For lab data which exceed limit of detection, e.g. data reported as >8 or >10, the threshold value 8 or 10 will be used in the summary descriptive statistics, and original reported data will be listed in the listings.

2.1.4 Subgroup definitions

The subgroups are defined based on subjects' status at screening (if screening value is not collected, Hour 0 value will be used).

Key subgroups that will be considered in subgroup analyses of the primary and key secondary endpoints are listed in Table 2-3.

Table 2-3 Specification of key subgroups at screening

Variable	Source	Categories
Age group	Derived	<65, ≥65; <75, ≥75 years
Sex	eCRF	Male, Female
[redacted]		
Preserved ejection fractions/Reduced ejection fractions (PEF/REF) (most recent value in last 12 months)	Derived	<50%, ≥50%; <40%, ≥40% (CRF: heart failure and diabetes history)
Prior history of heart failure	eCRF	Yes, No (CRF: heart failure and diabetes history)
[redacted]		

Table 2-4 Specification of additional subgroups at screening

Variable	Source	Categories
Systolic blood pressure	Derived	<140, ≥140 mmHg
Heart rate	Derived	< 80, ≥ 80 bpm
History of Ischemic heart disease	eCRF	Yes, No (CRF: heart failure and diabetes history)
History of diabetic mellitus	eCRF	Yes, No (CRF: heart failure and diabetes history)
History of atrial fibrillation or flutter	eCRF	Yes, No (CRF: protocol solicited medical history)
Time from presentation to randomization	Derived	<2, 2-10, >10 hours
Intravenous (IV) nitrate use at randomization	eCRF	Yes, No
Lymphocytes	Local Lab, Derived	≤12, >12%
eGFR	Local Lab, Derived	<30, ≥30; <50, ≥50; <60, ≥60 mL/min/1.73 m2

2.1.5 Subject disposition

The number of subjects randomized (RAN) and included in each analysis population (FAS, PPS, and SAF) will be presented by treatment group. The number of subjects screened, completed and discontinued from screen phase, and the reasons for screen failures will be provided. The number and percentage of subjects in the FAS who completed, discontinued the study, and the reason for discontinuation will be presented for each treatment group and overall (treatment groups combined).

The number of subjects screened and randomized per country will be presented for FAS.

For each subject, the length of follow-up (days) will be computed by subtracting the study drug initiation date (or the randomization date for subjects not treated with study drug) from the end of study date [redacted].

[redacted]

2.1.6 Background and demographic characteristics

Subject demographics (age, sex, ethnicity, race, weight, height and BMI) and baseline characteristics will be summarized for the FAS populations. Screening BNP, NT pro-BNP and estimated eGFR, laboratory values as reported by the investigator will be summarized with standard descriptive statistics. The number and percentage of subjects who were hospitalized for heart failure in the past year and subjects on IV nitrates at the time of randomization will both be presented.

For baseline comparability, treatment groups will be compared using the chi-squared test for categorical variables and using t-test for continuous variables. The p-values will be provided for descriptive purpose and will not be considered to define any formal basis for determining factors to be included in statistical models. If an imbalance of treatment groups with respect to some variables does occur, supplemental analyses with addition of these variables in model may be performed to assess the potential impact on efficacy as appropriate.

In addition, violations of inclusion and exclusion criteria and other protocol deviations will be listed. Major protocol deviations resulting in subject exclusion from any analysis (Table 2-1) will be summarized by treatment group for RAN population.

2.1.7 Medical history

Medical history data will be summarized by treatment group for FAS.

Protocol solicited medical history

The number and percentage of subjects will be presented for each protocol solicited event as pre-printed list in eCRFs.

Heart failure and diabetes history

The number and percentage of subjects including but not limited to who had history of heart failure, New York Heart Association (NYHA) class, primary heart failure etiology (ischemic/non-ischemic), who were hospitalized for heart failure, ejection fraction (<40%, ≥40%; <50%, ≥50%), who were on heart transplant list, and who had history of diabetes mellitus will be presented for FAS. The number of HF hospitalization in the past year and ejection fraction (%) will also be presented.

Other medical history

[redacted] The number and percentage of subjects with each medical condition will be provided by treatment group for the FAS.

2.1.8 Other subject history information

Drug abuse, smoking, and alcohol histories will also be summarized for the FAS by presenting the number and percentage of subjects for each pre-printed questionnaire.

2.1.9 Study medication

In general, study drug administration details will be summarized for the FAS and SAF populations. This will include time from presentation to randomization (hours), time from first IV loop diuretic for the current AHF episode to randomization (hours), time from earlier of presentation to hospital or first IV loop diuretic to randomization (hours), on IV nitrates at randomization (yes/no), time from randomization to study drug administration (hours), study drug administered on either day (yes/no), reason study drug not administered, actual study drug received, and the number of days infused (one or two).

The duration of study drug administration (in hours) and the total volume of study drug administered (estimated from the total time and rate of infusion) will be summarized. In addition, the number of subjects whose study medication dose was lowered or discontinued prematurely, and the reasons for discontinuation, including dose decreased due to blood pressure decrease (yes/no) will be summarized by treatment group.

2.1.10 Concomitant medications, surgical and medical procedures

Unless specified, the use of all concomitant medications will be summarized using the SAF population.

Major cardiovascular and non-cardiovascular classes of medication taken by a subject approximately 30 days prior to study drug initiation and on a daily basis while hospitalized through Day 5, at Discharge, and at Day 14 and other follow-up visits will be recorded. At each visit, only those medications currently being taken or those were taken within 24 hours prior to the given visit will be collected. These medications are predefined by the code lists and will be collected in each of following eCRFs:

- IV loop diuretic medications for heart failure (collected since initial presentation of HF)
- IV medications other than loop diuretics(collected since initial presentation of HF)
- Other non-IV prior medications (30 day prior to screening, within 24 hours at each visit)
- Other prior and concomitant medications

IV loop diuretic medications

IV loop diuretic medications for heart failure include Bumetanide, Etacrynic acid, Ethacrynic acid, Furosemide, Torasemide, Torsemide and Piretanide.

The number and percentage of subjects using each medication will be summarized by protocol scheduled time point. Each time point reflects medication use within the preceding 24 hours.

The total daily dose for each medication will also be presented at each time point. In addition, cumulative use (total dose as the sum from Day 1) at 48 hours (24 hours/Day 1 and 48 hours/Day 2), and at Day 5 (from 24 hours/Day 1 to Day 5) in furosemide equivalent (mg) will be summarized. The calculation of furosemide equivalents (mg) for Bumetanide, Etacrynic acid/Ethacrynic acid ,Torsemide/Torsemide acid and Piretanide are actual dose (mg) multiplied by constant 40, 0.8, 2, or 6.67, respectively.

Doses will be imputed as zero for study days where no use was reported. [redacted]

IV medications other than loop diuretics

Other IV medications include not limited to Dopamine, Dobutamine, Milirinone, Enoximone, Norepinephrine, Epinephrine, Nesiritide, Levosimendan, Isosorbide Dinitrate, Nitroglycerin, Nitroprusside, Phenylephrine, Caperitide and Other (including but not limited to Danshen).

The number of subjects using each medication will be presented in the same way as IV loop diuretic medications.

The average dose over the time administered of the medication will be presented separately for each medication at each reported time point. No equivalent dose unit among other IV medications will be converted.

Note that the frequency (number and percentage) of subjects using IV loop diuretic and vasoactive agents through Day 5 is an efficacy endpoint and it's discussed in Section 2.2.3.

Other non-IV prior medications & other non-IV medications

The number and percentage of subjects using other non-IV medications will be summarized for each of the time periods for general, ACE inhibitor (ACEi), angiotensin inhibitor (ARB), ACEi or ARB, beta blocker, PO loop diuretics, other PO diuretics, calcium channel blockers, aldosterone antagonist and cardiac glycosides.

The number (%) of subjects who have these medications initiated during their index hospitalization and subjects who have these medications increased during the period following discharge from the index hospitalization will also be presented.

The average daily dose administered will be summarized at each time point for each medication within the loop diuretics, ACEi, ARB, beta blocker, aldosterone antagonist, ARNi and direct renin inhibitor.

Other non-IV medications taken prior to and after the start of study drug will be presented by the ATC and preferred terms.

Other prior and concomitant medications

All other concomitant medications (not included in above classes) and significant non-drug therapies, 30 days prior to screening and ongoing basis until subjects completed or discontinued from the study, will be summarized respectively by ATC therapeutic class, preferred term, and treatment group for the SAF population.

Alternative medicine or herbal medicine will also be presented for the SAF population.

2.2 Efficacy evaluation

The Full Analysis Set (FAS) is the primary analysis population for efficacy analyses, following the intention-to-treat principle. Per-protocol (PPS) population will be used in the supportive analysis for primary and key secondary efficacy parameters (Table 2-5).

Table 2-5 List of efficacy endpoints and analyses

Endpoint	Analysis set	Analysis method
Primary efficacy endpoint (α=0.025, 1-sided)		
1 Trichotomous composite (success, failure, no change)	FAS/PPS	Ordered logistic-regression (proportional odds) model that includes treatment and [redacted] as factors (***primary analysis is on FAS***)
	FAS	Ordered logistic-regression model including treatment only (*sensitivity analysis 1*)
	FAS	van Elteren extension of the Wilcoxon test stratified by [redacted] (*sensitivity analysis 2*)
Secondary efficacy endpoints (if primary endpoint is significant, then test in the order listed below at α=0.05 each)		
1 Time (hours) to WHF through Day 5	FAS/PPS	Gehan's generalized Wilcoxon test
2 Time (days) to CV death through Day 180	FAS/PPS	Kaplan-Meier estimates and log-rank test
3 Time (days) to all-cause death through Day 180.	FAS	Kaplan-Meier estimates and log-rank test
Other efficacy endpoints (at α=0.05, no multiplicity adjustment)		
1 Time (hours) to moderate or marked improvements in dyspnea by Likert scale through Day 5	FAS	Wilcoxon rank-sum test
2 Dyspnea by VAS-AUC changes (from baseline) through Day 5	FAS	ANCOVA
3 Length of stay (# of days) in index ICU/CCU	FAS	Wilcoxon rank-sum test
4 Renal dysfunction or worsening of renal function through Day 5	FAS	chi-squared test
5 Time (days) to re-hospitalization due to HF and renal failure through Day 180	FAS	Kaplan-Meier estimates and log rank test
6 Time (days) to CV death or re-hospitalization due to HF/RF through Day 180	FAS	Kaplan-Meier estimates and log rank test
7 Time to in-hospital worsening heart failure through Day 5 defined by symptoms and signs		Kaplan-Meier estimates and log rank test
8 Use of loop diuretics and vasoactive agents through Day 5	FAS	chi-squared test
9 Changes in cardio-renal biomarkers (Day 2 and Day 5)	FAS	Summary statistics, see Section 2.2.3

2.2.1 Primary efficacy variable and analyses

2.2.1.1 Primary efficacy variable

The primary efficacy variable is the trichotomous clinical composite endpoint based on

(i) treatment 'success': as of congestion improvement of dyspnea in Likert (moderately and markedly improvement) AND at least 2 points improvement for at least 2 out of 4 signs and symptoms variables (orthopnea, edema, rales and JVP) at Day 2

(ii) treatment 'failure': as of in-hospital worsening heart failure (WHF), all causes of death, or re-hospitalization due to heart failure (HF) or renal failure (RF) through Day 5

(iii) no change: neither of (i) or (ii)

If a patient meets (i) and (ii), he will only be counted as 'failure' (ii).

For missing Day 2 Likert or signs and symptoms, the previous available post baseline values will be used.

[redacted]

2.2.1.3 Handling of missing values/censoring/discontinuations

The components of trichotomous clinical composite endpoint are all categorical variables. Missing categorical value will be replaced with the last preceding non-missing value. These will include dyspnea Likert scale at Day 2, four signs and symptoms at Day 2 and worsening heart failure through Day 5. In the evaluation of treatment success for the four signs and symptoms, the change from baseline will need to be calculated. If there is a missing baseline value, say a subject with missing JVP at baseline, this subject's change of JVP at Day 2 will be set as 0-point improvement, i.e., will not be contributed as 2-point improvement for JVP.

[redacted]

[redacted]

2.2.2 Secondary efficacy variables and analyses

There are three secondary variables:

- WHF through Day 5
- CV death through Day 180
- All-cause death through Day 180

All of these secondary variables are time-to-event variables. The hypothesis will be tested based on the FAS at a significance level of 0.025 (one-sided).

The three secondary variables will be tested using hierarchical testing procedure in controlling overall α of 0.05 (Bretz et al 2009). If primary endpoint is significant, WHF will be tested first at α of 0.05. If WHF is significant at the same α level then will proceed to test CV death and only if CV death is significant then will test all-cause death at α of 0.05.

WHF through Day 5

Time to WHF through Day 5 will be analyzed using Gehan's generalized Wilcoxon test. Subjects died in the 5-day period will be considered as having WHF event. Event date and time will be used at calculating the time to event for better precision, and convert it back to days.

Time-to-event (days) = (first event date/time– baseline date/time),

For those subjects without events, the censoring date will be the earlier of

- Hour 120 (5 days)
- The last date at which the vital status was known, including last visit or contact

Kaplan-Meier curves will be presented graphically by treatment group and Kaplan-Meier estimates for selected time points with 95% confidence intervals will be tabulated.

In addition, analyses will be performed using a modified definition of WHF, e.g. subjects with (1) worsening signs AND symptoms or (2) IV intervention and/or mechanical support. Another sensitivity analyses that exclude subjects whose only symptom is abdominal bloating/discomfort or fatigue/decreased exercise tolerance will also be provided.

CV death through Day 180

CV death will be analyzed with a log-rank test. The time-to-event will be calculated days by subtracting the randomization date from the event date. Number and percentage of subjects who died from cardiovascular reasons based on the number of subjects in the population as denominator will be provided by treatment group. The hazard ratio (relative risk) and its associated two-sided 95% confidence interval will be estimated based on a Cox proportional hazards model with treatment assignment as a factor.

In addition, the Kaplan-Meier estimates of the survival functions for each treatment group will be plotted. The Kaplan-Meier estimates of the cumulative event rate will also be presented in tables by treatment group for each day and also by time interval.

For CV death, a patient without an event will be censored at the earlier of:

- Date of death for non-CV related causes
- The last date at which the vital status was known, including last visit or contact
- Day 180

An adjudicated death with unknown cause will be classified as a CV death. Similarly, investigator suspected deaths of unknown cause will be classified as CV deaths.

All-cause death through Day 180

All-cause death through Day 180 will be analyzed similarly to CV death through Day 180. A patient without an event will be censored at the last date at which the vital status was known (including last visit or contact) or at Day 180.

Of note, CV death and all-cause death will be two efficacy variables for bridging with other global studies.

2.2.3 Other efficacy variables and analyses

Other efficacy variables are listed along with primary and secondary endpoints in Table 2-5.

Time to moderate or marked improvements in dyspnea assessed by Likert scale through Day 5

Subject-reported dyspnea by Likert scale will be summarized by frequency table at each scheduled time point through Day 5 as defined in protocol. Time to moderately or markedly improvement comparing with baseline will be analyzed by Wilcoxon rank-sum test, based on discrete scheduled hours.

Since the assessment only occurs on the scheduled visits, the nominal scheduled day will be used directly to determine the time to event.

For those subjects without an event, the censoring date will be the earlier of:

- Day 6
- The last date at which the vital status was known, including last visit or contact

AUC of changes from baseline in patient-reported dyspnea by VAS scale through Day 5

The area under the curve of the change from baseline in dyspnea VAS scale (VAS AUC) will be computed by trapezoidal rule after applying the data handling conventions and computations as described below.

Subjects who die or have a worsening heart failure event (either during the index hospital or rehospitalization due to heart failure) will have their score imputed to be the worst observed score in any subject at any time point in the respective analysis population carried forward from the day of death or WHF regardless of whether their original data are missing.

For post-baseline visual analog scale (VAS) values otherwise missing, a missing score will be imputed using linear interpolation between the last preceding and first following non-missing values. If no following non-missing value is available, the last available preceding value will be carried forward. A missing baseline score will be imputed as the earliest, non-missing score within 24 hours for the subject minus the average change from baseline in the study population to that time point; post-baseline scores for subjects for whom a missing baseline cannot be thus imputed will be included in the analysis as no change from baseline. Except for subjects who die or who experience WHF, subjects who are missing all post-baseline dyspnea VAS scores will be included in the analysis as having no change from baseline at any time point.

Descriptive statistics of the area under the curve for the dyspnea VAS change from baseline scores will be presented at each time interval of interest by treatment group. Treatment groups will be compared by presenting the mean difference and corresponding 95% CI and using ANCOVA model with baseline VAS as a covariate.

For those subjects who have at least one post-baseline assessment, their AUC will be approximated by $\sum^{m}_{k=2} w_k \tilde{y}_k$, where $\tilde{y}_k = 0.5(y_k + y_{k-1})$, $w_k = (t_k - t_{k-1})/(t_m - t_1)$, and y_j is the change-from-baseline in dyspnea VAS value at time t_j, for $j = 1, \ldots, m$, in which $t_1 < \ldots < t_m$. m is the number of measurements for a subject and t_1 is time of the baseline, that is, $t_1 = 0$ and $y_1 = 0$. The scheduled time will be used in the calculation.

In addition, descriptive statistics for the baseline values, the post-baseline values, and the change from baseline value will be provided at each scheduled time point by treatment group.

Length of stay in index ICU/CCU

The length (days) of stay in ICU/CCU will be obtained from eCRF page directly. If the information is missing in eCRF page or subjects who die during the initial hospitalization, the maximum number of days in the ICU/CCU over all subjects in FAS will be assigned. It will be analyzed using a Wilcoxon rank sum test.

Renal dysfunction or in-hospital worsening of renal function through Day 5

Worsening renal function is defined as an increase in serum creatinine of ≥ 0.3 mg/L (27 μmol/L), from the values measured at baseline. A subject who meets the criteria at any time during the 5 days after infusion will be counted once; the chi-squared test will be performed for comparing two treatments.

Number and percentage of subjects will be displayed by treatment and visit for the following category: ≥ 0.3 mg/L increase in serum creatinine.

Additional summaries of serum creatinine and eGFR are discussed in Section 2.4.2.2, Clinical chemistry.

Time to CV death or rehospitalization due to heart failure (HF)/renal failure (RF) through Day 180

Time-to-event (in days) will be calculated using time of first event of adjudicated CV death or adjudicated rehospitalization due to HF/RF minus time of randomization. If the reason for adjudicated death or rehospitalization is unknown, it will be considered as the event. The analysis and censoring method will be performed similarly to time-to-CV death.

Time to rehospitalization due to heart failure (HF) or renal failure (RF) through Day 180

Time-to-event (in days) will be calculated using time of first event of adjudicated rehospitalization due to HF or RF minus time of randomization. If the reason for adjudicated rehospitalization is unknown, it will be considered as the event.

The analysis and censoring method will be performed similarly to time-to-all-cause-death.

Due to competing risk between death and rehospitalization, the difference in mortality between the treatment groups may mislead the results. In this case, the composite endpoint of Time to CV death or rehospitalization due to heart failure (HF)/renal failure (RF) through Day 180 provides more reliable results.

Time to in-hospital worsening heart failure through Day 5 by symptoms and signs

Time-to-event (in days) will be calculated using time of first event of in-hospital worsening heart failure through Day 5. The worsening heart failure is defined by symptoms only, signs only, and by both symptoms and signs. The analysis and censoring method will be performed similarly to time-to-WHF.

Use of IV loop diuretic and vasoactive agents through Day 5

The subjects with intravenous diuretic and vasoactive therapy (yes/no) during 5 days after study medication infusion will be analyzed using chi-squared test.

The medications of interest are collected in two eCRF pages: “IV loop diuretic medications for heart failure” and “IV medications other than loop diuretics” (Section 2.1.10). The summaries of doses for those medications are also addressed in Section 2.1.10.

Change from baseline in cardio-renal biomarkers through Day 2 and Day 5

Biomarkers related to cardic and renal function/injury including but not limited to high sensitivity troponin T, NT-proBNP, and Cystatin C will be collected at baseline, at 48 hours (Day 2) and 120 hours (Day 5) post infusion. This list of potential biomarkers may be changed or expanded further as it is recognized that more relevant or novel biomarkers may be discovered.

Details are described in the separate biomarker statistical analysis plan.

2.3 Pharmacokinetic evaluations

All subjects with at least one available valid (i.e. not flagged for exclusion) PK concentration measurement, who received serelaxin infusion and experienced no protocol deviations with relevant impact on PK data will be included in the pharmacokinetic (PK) data analysis. Steady state concentration (Css) will be estimated based on the serum concentration determined at the 48 hour time point prior to the end of infusion. Systemic clearance will be calculated using the rate of infusion and Css for each subject as indicated below:

Clearance (mL/hr/kg) = Rate of infusion (µg/kg/hr) /Css (µg/mL)

Where the rate of infusion is the actual dosing rate calculated as below for each individual subject:

$$\underset{\mu g/kg/day}{Rate} = \frac{VolRLX}{VolRLX+VolSol} \times \underset{\mu g/mL}{ConcRLX} \times \underset{mL/hr}{Flow} \times \underset{hrs/day}{24} / \underset{kg}{Weight}$$

where VolRLX is the volume of serelaxin drawn from the vials and added to a volume VolSol of the solution contained in the infusion bag, ConRLX is the concentration of the serelaxin stock solution in the vial (1000 µg/mL), Flow is the flow rate of the solution from the infusion bag, and Weight is the body weight measured at Day 1 pre-dose baseline. Since only sparse PK time points for serelaxin serum concentration will be collected, no other PK parameters will be determined in this study.

Missing values or those below the assay LLOQ (BLQ) will be indicated in the data listings and treated as zero for PK parameter calculations and presentation.

Descriptive statistics for serelaxin concentrations will be listed by time points (for baseline and 48hr samples only) to include arithmetic mean, geometric mean with its corresponding 90% confidence interval, median, SD, CV, min and max. A value of "0" will be used for samples that are BLQ in these calculations and therefore be excluded from geometric mean calculations. Descriptive statistics include arithmetic mean, geometric mean with its corresponding 90% confidence interval, median, SD, CV, min and max will also be provided for the two PK parameters, Css and clearance (CL).

Subgroup analysis on Css and CL to assess the potential effects of various extrinsic and intrinsic factors on the PK of serelaxin may be performed as appropriate. These factors may include but not limited to renal function (e.g. eGFR), age, gender, race or ethnicity, country, concomitant medications, etc.

Serelaxin serum concentration will be assayed in Day 60 samples as well, in order to demonstrate the lack of drug interference with the immunogenicity assay. This data will be included only as part of the immunogenicity results but not in the PK results (see Section 2.6.3).

2.4 Safety evaluation

Safety evaluations including AE, SAE, laboratory, vital sign, confirmed blood pressure decreases, ECG etc. will be reported for safety population (SAF) by treatment as received.

2.4.1 Adverse events

2.4.1.1 AEs and SAEs

The incidence of AEs recorded through Day 5 and the incidence of SAEs recorded through Day 14 will be presented for the Safety population. Incident AEs will be considered those AEs with an onset date and time *after* the initiation of study drug. Adverse events with an onset between informed consent and study drug initiation will be listed separately. Adverse events will be coded using latest version of MedDRA. All reported AEs will be summarized by system organ class (SOC) and preferred term (PT) by treatment groups. Serious AEs (SAEs) will be summarized similarly. Percentages will be based on the number of subjects in the SAF.

In addition, SAEs will be summarized by time period of onset: from study drug initiation up to and including Day 5, or from Day 6 up to and including Day 14. All AEs through Day 14 will also be provided. After Day 14 only suspected serious adverse events will be collected and reported to Novartis. Additionally, a summary of AEs by PT and severity, using the worst reported severity grade for each event for the subject, will be provided. For analysis purposes, if the relationship to study drug is unknown or missing, the AE will be considered to be drug-related.

All study-drug-related AEs, AEs with an outcome of death, AEs leading to discontinuation of treatment, and study-drug-related SAEs, SAEs with an outcome of death, and SAEs leading to study drug discontinuation will be summarized by percentages and frequencies. For the analysis by time period of onset, the percentages will be based on the number of subjects at risk for AEs at the beginning of the period in the Safety Set. For each adverse event listed, the verbatim and coded terms, the corresponding severity, relationship to study drug, seriousness (yes/no), and start and stop date and time of the event will be specified and displayed by treatment group and subject number.

Detailed AE listings will also be provided.

2.4.1.2 Selected AEs

AEs representing identified and potential safety risk in addition to events of interest will be grouped. The incidence (absolute and relative frequency) of each potential risk will be summarized by treatment group. Odds ratios and associated 95% confidence intervals comparing serelaxin with placebo will be presented using the Mantel-Haenszel test. In addition, subjects experiencing each of these risks will be listed. Such safety risks and events of interest are displayed in below table.

Table 2-6 Safety risk and event of interest search specification

Type of Risk	Specification
Safety risk	
Hypotension	NMQ ‘Hypotension’ (Broad)
Hemoglobin/ hematocrit transient decrease	SMQ ‘Haemorrhages’ (Broad) HLGT ‘Anaemias nonhaemolytic and marrow depression’ SMQ ‘Haematopoietic erythropenia’ (Broad)
Fetotoxicity and teratogenicity	SMQ ’Pregnancy and neonatal topics’ (Broad)
Event of interest	
Hypokalemia	NMQ ‘Hypokalemia’ (Broad)
Menorrhagia/Metrorrhagia	HLGT ‘Menstrual cycle and uterine bleeding disorders’
Potential promotion of cancers	SMQ ‘Malignancies’ (Broad)
Immunogenicity/Hypersensitivity	SMQ ‘Hypersensitivity’ (Broad) SMQ ‘Angioedema’(Broad) SMQ ‘Anaphylactic reaction’(Broad) SOC ‘Immune system disorders’ HLGT ‘Immunology and allergy investigations’ HLGT ‘Administration site reactions’

Type of Risk	Specification
	SMQ 'Severe cutaneous adverse reactions'(Broad)
Cardiac failure	SMQ 'Cardiac failure' (Broad)
QT prolongation	SMQ 'Torsade de pointes/QT prolongation' (Broad) SMQ 'Cardiac arrhythmias' (Broad)
Thromboembolic events	SMQ 'Embolic and thrombotic events'
Renal impairment	SMQ 'Acute renal failure' (Broad)
Respiratory failure	SMQ 'Respiratory failure' (Broad)
Hepatotoxicity	SMQ 'Drug related hepatic disorders – comprehensive search' (Broad)

AEs with an onset between informed consent and study drug initiation will be listed.

[redacted]

2.4.2 Laboratory data

According to protocol, a central laboratory will be used for the analysis of all baseline and post-baseline specimens collected, except for hematology and clinical chemistry at screening, it will be done locally and the result will be entered into eCRF.

2.4.2.1 Hematology

The following hematology tests will be measured locally at screening and centrally at baseline and post-baseline: RBC, hemoglobin, hematocrit, platelet, and white blood cell count, including differentials.

2.4.2.2 Clinical chemistry

The following serum chemistry tests will be measured locally at screening and centrally at baseline and post-baseline: sodium, potassium, glucose, blood urea nitrogen (BUN) or urea, AST, ALT, alkaline phosphatase, total bilirubin, and creatinine. In addition, BNP/NT pro-BNP, eGFR and hemoglobinA1c will be measured locally at screening only.

Hematology and clinical chemistry data from central lab will be summarized for safety population by presenting shift tables using extended normal ranges (baseline to most extreme post-baseline value), summary statistics of raw data and change from baseline (means, medians, standard deviations, 1st and 3rd quartiles (Q1 and Q3), and by flagging of notable values in data listings. Shift tables are based on the cross-classification of the number of subjects with clinical laboratory values below (low), within (normal), or above (high) normal ranges for each laboratory test.

The number and percentage of subjects with clinically notable laboratory results after baseline will be presented. Clinically notable laboratory values are defined below.

Table 2-7 Clinically notable laboratory abnormalities for selected tests

Parameter	Criteria (based on a percent change from baseline)
Hematology	
RBC count	>50% increase, >25% decrease
Hemoglobin	>50% increase, >25% decrease
Hematocrit	>50% increase, >25% decrease
WBC count	>100% increase, >50% decrease
Platelet count	>100% increase, >50% decrease
Blood Chemistry	
ALT (SGPT)	defined in Section 2.4.7.1 (liver toxicity)
AST (SGOT)	defined in Section 2.4.7.1 (liver toxicity)
BUN	>100% increase
Creatinine	>100% increase
Total bilirubin (TBL)	defined in Section 2.4.7.1 (liver toxicity)
Alkaline phosphatase (ALP)	defined in Section 2.4.7.1 (liver toxicity)
Potassium	>25% increase, >25% decrease
Calcium	>20% increase, >20% decrease
Uric acid	>100% increase

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ULN: upper limit of normal range

The data from local and central laboratory will be presented separately. All laboratory data from central lab will be listed for each subject. Laboratory values that are outside the normal ranges will be flagged high (H) or low (L). Data from local laboratory will be listed separately by lab.

Creatinine and eGFR

eGFR will be calculated using simplified Modification of Diet in Renal Disease (sMDRD).

The proportion of subjects with >= 0.3 mg/dL increase, >=0.5 mg/dL increase, >= 1.0 mg/dL increase, and >50% increase from baseline in serum creatinine levels at each time point, and at any time point, will be presented.

The proportion of subjects with decreases from baseline eGFR of >25%, >40%, >50%, and >30 mL/min/1.73 m^2 at each time point, and at any time point, will be presented.

In addition, worsening renal function based on serum creatinine and plasma cystatin-C values as one of efficacy variables is defined and discussed in Section 2.2.3.

Blood sample for hepatitis serology

A blood sample will be collected at baseline and stored at the central laboratory for hepatitis serology analysis in case of a significant liver event (refer to Section 2.4.7).

2.4.3 Vital signs

For vital signs, descriptive statistics will be provided for values and change from baseline at each assessment time point for pulse, respiratory rate, body temperature, and weight. Treatment groups will be compared for changes from baseline using t-test.

Blood pressure and pulse during 48 hours drug infusion and 48 hours post infusion will also be listed.

2.4.4 Electrocardiogram (ECG)

[redacted]

The following quantitative variables will be summarized: ventricular rate, PR interval, QRS duration, QT interval, and corrected QT interval (QTc).

The number and percentage of subjects with the following criterion will be presented.

- QT > 500 msec
- QT > 480 msec
- QT > 450 msec
- QT increases from baseline >= 30 msec
- QT increases from baseline >= 60 msec
- PR > 200 and <= 220 msec
- PR > 220 msec
- PR increases from baseline > 25% (PR > 200 msec)
- QRS > 110 and <= 120 msec
- QRS > 120 msec
- QRS changes from baseline > 25% (QRS > 110 msec)

- Heart rate >100 beats per minute
- Heart rate < 50 beats per minute

QT data will be analyzed for the Fridericia (primary) corrections.

In addition, shift tables comparing baseline ECG results (normal, abnormal, not available, total) with the maximum on-study result (normal, abnormal, not available, total) will be provided for each variable.

A listing of all newly occurring or worsening abnormalities will be provided, as well as a by-subject listing of all quantitative ECG parameters.

2.4.5 Confirmed blood pressure decreases

The number and proportion of subjects who experience a confirmed blood pressure decrease event during study drug administration will be provided. Among subjects who experience a confirmed blood pressure decrease event, the events will be further characterized with respect to impact on study drug administration (dose decreased or study drug discontinued), the timing of event onset relative to study drug initiation, the magnitude of the blood pressure decrease at its onset and the trough blood pressure recorded, the blood pressure measurements after study drug discontinuation, outcome, treatment(s) required, and whether considered symptomatic, i.e. an AE. Summaries will be provided separately for those confirmed events that resulted in study drug dose reduction, and those that resulted in study drug discontinuation. The possible interaction between the effect of serelaxin and the effect of IV nitrate administration within the first 48 hours will be examined.

Course of blood pressure after the occurrence of first CBPD up to Day 5 will be presented.

To further investigate the impact of CBPD, the following analyses will be performed.

- Baseline characteristics by CBPD subgroup (yes/no).
- Concomitant medications by CBPD subgroup (yes/no).
- AEs associated with the occurrence of the CBPD.

2.4.6 Pregnancy test results

Positive results will be presented in listing.

2.4.7 Liver toxicity function and liver events

Liver event and laboratory trigger definitions are defined in Appendix 2 of protocol.

2.4.7.1 Liver toxicity function

The liver toxicity (LFT abnormality) will be identified with the Adverse Event (jaundice and "Drug-related hepatic disorders - severe events only" SMQ AE), and the laboratory parameters mainly AST (aspartate aminotransferase; also known as SGOT), ALT (alanine aminotransferase; also known as SGPT), ALP (alkaline phosphatase) and TBL (total bilirubin; conjugated (direct) and unconjugated (indirect) bilirubin).

The standard SMQ-PT table and odds ratio will be used to provide the number and percentage of subjects with hepatic disorders as mentioned in Section 2.4.1.2, Selected AEs.

The laboratory parameters for liver function will be shown in the laboratory standard tables as discussed in Section 2.4.2.2.

In addition, summary tables will be provided on the number and percentage of subjects who meet the following criteria at post baseline.

Table 2-8 Criteria for evaluating liver toxicity

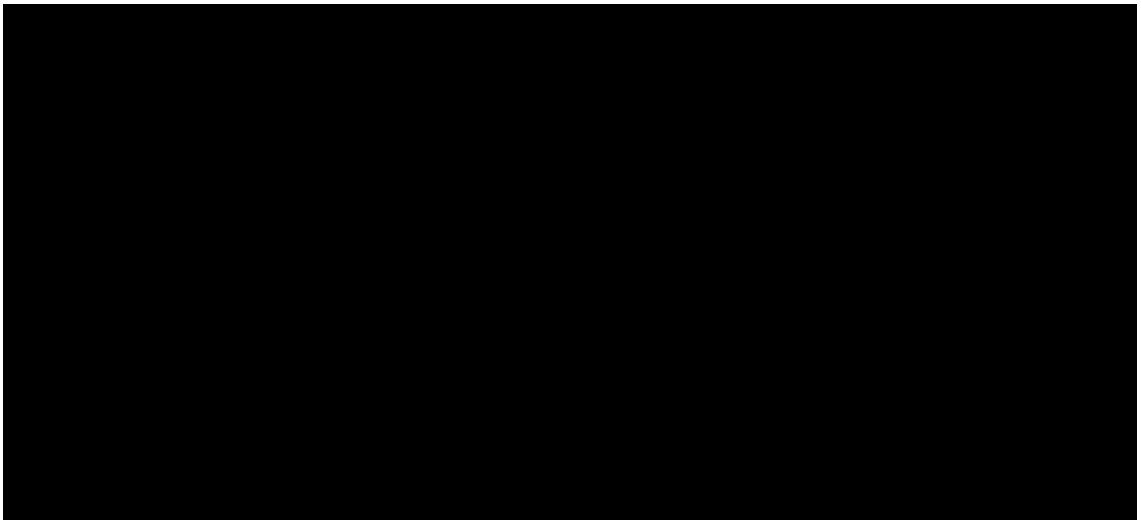
Parameter	Criterion
ALT or AST	ALT or AST > 3xULN
	ALT or AST > 5xULN
	ALT or AST > 8xULN
	ALT or AST > 10xULN
Hy's category	ALT or AST >3x ULN and TBL >1.5x ULN
	ALT or AST > 3xULN & TBL > 2xULN
	ALT or AST > 5xULN & TBL > 2xULN
	ALT or AST > 8xULN & TBL > 2xULN
	ALT or AST > 10xULN & TBL > 2xULN
	ALT or AST > 3xULN & TBL > 2xULN & ALP < 2xULN
TBL&ALP	TBL >1.5x ULN and ALP >2x ULN
	TBL >2x ULN and ALP >2x ULN
Isolated TBL	TBL >1.5x ULN & ALT and AST $\leq$3x ULN and ALP $\leq$2x ULN
	TBL >2x ULN & ALT and AST $\leq$3x ULN and ALP $\leq$2x ULN
	TBL >3x ULN & ALT/AST $\leq$3x ULN and ALP $\leq$2x ULN
Isolated ALP	ALP >1.5x ULN & ALT and AST $\leq$3x ULN and TBL $\leq$1.5x ULN
	ALP >2x ULN & ALT and AST $\leq$3x ULN and TBL $\leq$1.5x ULN
	ALP >3x ULN & ALT and AST $\leq$3x ULN and TBL $\leq$1.5x ULN
	ALP >5x ULN & ALT and AST $\leq$3x ULN and TBL $\leq$1.5x ULN

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal.

Plots showing the relationship between TBL and ALT or AST (eDish plot) will also be provided.

2.4.7.2 Liver events

For subjects who are identified with a liver event, additional information will be collected as pre-printed in the following eCRFs:



2.5 Interim analysis

There will be no formal efficacy interim analysis.

However to monitor subject safety data on a regular basis during the course of the study, an external independent data monitoring committee (DMC) has been established and will review safety data two times a year as planned in the DMC charter. DMC may request additional safety data reviews as needed. Such safety data reviews do not inflate the type I error for the primary efficacy hypothesis testing and thus require no multiplicity adjustments.

2.6 Other topics

2.6.1 Resource utilization

Summary statistics on hospital length of stay (days) for the index hospitalization and length of ICU/CCU stay for the index and subsequent hospitalizations, as well as procedures rendered during these hospitalizations will be provided by treatment group for FAS.

2.6.2 Health-related Quality of Life

Health related quality of life assessments will not be collected in this study.

2.6.3 Immunogenicity

Anti-serelaxin antibodies will be measured in serum samples collected at the Day 60 time point. To account for individual variability in background of each subject and to check for endogenous interference and/or pre-existing anti-serelaxin antibodies, anti-serelaxin antibodies will also be measured at baseline. The analysis will be done for all subjects who received any study treatment and who had an anti-serelaxin antibody measurement performed.

Results of the anti-serelaxin antibody assays (including anti-serelaxin antibody positive/negative, titer, and neutralizing antibody positive/negative) will be listed by treatment group, subject and sampling time. Incidence rate of positive anti-serelaxin antibodies and neutralizing antibodies will be computed and summarized by treatment group and sampling time.

2.7 Determination of sample size

A sample size of 1,520 AHF subjects will need to be randomized 1:1 to serelaxin and placebo. This study will have 80% power at a two-sided type-I error of 5% to detect a distributional shift with a common odds ratio of 0.75 between the distributions of placebo and serelaxin as presented in the table below, where the distribution of categories of the clinical composite endpoint in placebo were based on data from RELAX-AHF (RLXN.C.003).

[redacted]

3 References

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2. Levey AS, Coresh J, Balk E, et al (2003) National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. Ann Intern Med; 139:137-147.
3. Metra M, Ponikowski P, Cotter G, Davison BA, Felker GM, Filippatos G, Greenberg BH, Tsushung HA, Severin T, Unemori E, Voors AA, Teerlink JR (2013). Effects of serelaxin in subgroups of patients with acute heart failure: results from RELAX-AHF. European Heart Journal;34:3128-3136.