Reasanz™ (serelaxin)

Briefing Document

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Abbreviations	Definitions
Ab	antibody
AC	arterial compliance
ACEi	angiotensin-converting enzyme inhibitors
ADR	adverse drug reactions
AE	adverse event
AHF	acute heart failure
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ARBs	angiotensin receptor blockers
AST	aspartate aminotransferase
AUEC	area under the effect curve
AUC	area under the serum concentration time curve
AUCinf	area under the serum concentration-time curve from time zero to infinity
AUCinf/Dose	dose normalized AUCinf
BB	beta blockers
BL	baseline
BP	blood pressure
BNP	B-type natriuretic peptide
BUN	blood urea nitrogen
CAD	coronary artery disease
cAMP	3'-5'-cyclic adenosine monophosphate
CBPDE	confirmed blood pressure decrease events
CCU	cardiac/coronary care unit
CEC	clinical endpoint committee
CHF	chronic (congestive) heart failure
CI	confidence interval / cardiac index
CL	serum clearance
Cmax	observed maximum serum concentration following administration
CR	creatinine
CO	cardiac output

COPD	Chronic Obstructive Pulmonary Disease
CRF	case report form
CRT	cardiac resynchronization therapy
Css	serum concentration at steady state
CV	cardiovascular
DAOOH	days alive out of hospital
DBP	diastolic blood pressure
DDI	drug-drug interaction
DMC	data monitoring committee
DNA	deoxyribonucleic acid
DOE	dyspnea on exertion
DRE	disease-related events
ECG	electrocardiogram
ED	emergency department
EF	ejection fraction
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
ET	endothelin
ET-1	endothelin-1
ET_A	endothelin receptor type A
ET_B	endothelin receptor type B
FAS	full analysis set
FDA	Food and Drug Administration
FF	filtration fraction
GD	gestational day
GFR	glomerular filtration rate
HF	heart failure
HR	heart rate / hazard ratio
hERG	human Ether-à-go-go-Related Gene
hs-cTnT	high-sensitivity cardiac troponin T
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IHC	immuno-histochemistry
IL-6	interleukin 6

INSL	insulin-like peptides
ITT	intent-to-treat
IV	intravenous
JVP	jugular venous pressure
K-M	Kaplan-Meier
LFT	liver function test
LOCF	last observation carried forward
LOS / LoS	length of stay
LS	least squares
LV	left ventricular
LVEF	left ventricular ejection fraction
MAP	mean arterial pressure
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
mITT	modified intent-to-treat
mPAP	mean pulmonary artery pressure
MRA	mineralocorticoid receptor antagonist
NNT	number needed to treat
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
PAP	pulmonary arterial pressure
PBO	placebo
pc	post-conception
PCWP	pulmonary capillary wedge pressure
PD	pharmacodynamic
PI3K	phosphatidylinositol-3-kinase
PK	pharmacokinetic
PRO	patient reported outcome
PT	preferred term
PVR	pulmonary vascular resistance
RAP	right atrial pressure

RBC	red blood cell
RBF	renal blood flow
RF	renal failure
RLX/RLX030	serelaxin
RPF	renal plasma flow
RXFP	relaxin family peptide receptor
SAE	serious adverse event
SBP	systolic blood pressure
sc	subcutaneous
SD	standard deviation
SE	standard error
SOC	System Organ Class
SMQ	Standardized MedDRA Query
sMDRD	simplified modification of diet in renal disease
SUSAR	suspected unexpected serious adverse reactions
SVR	systemic vascular resistance
$T_{1/2}$	terminal half-life
TB	total bilirubin
TNF-α	tumor necrosis factor-alpha
TnT	troponin T
ULN	upper limit of normal
US	United States
VAS	visual analog scale
V_{ss}	volume of distribution at steady state
WHF	worsening of heart failure
WRF	worsening of renal function

1 Executive Summary

Serelaxin is a recombinant form of the human relaxin-2 hormone that is under consideration to improve the symptoms of acute heart failure through reduction of the rate of worsening heart failure. Serelaxin is formulated as a sterile solution to be administered via continuous intravenous (IV) infusion over 48 hours.

1.1 Background on Acute Heart Failure

(Section 2)

Acute heart failure (AHF) is a major and growing public health problem in the US with more than one million hospitalizations annually. AHF is defined as the rapid onset of, or change in, signs and symptoms of heart failure (HF), eg, breathlessness (dyspnea), orthopnea, paroxysmal nocturnal dyspnea, and fatigue requiring urgent therapy and hospitalization.Following initial stabilization, in-hospital worsening heart failure (WHF) occurs in 10-30% of hospitalized patients, reflecting a severe manifestation of the AHF symptom continuum, and is associated with a protracted in-hospital clinical course and increased risk of rehospitalization or death. In-hospital WHF is a distinct clinical event that represents the occurrence of treatment failure, and its prevention constitutes an important treatment goal.

Overall, AHF has a poor prognosis with approximately 4% in-hospital mortality, and post-hospitalization mortality rates that range from 20-35% at 1 year and 45-60% at 5 years, respectively. Hospital readmission rates are also high at 30% and 65% at 3-6 months and 1 year, respectively.

Despite the increasing prevalence and the poor prognosis, there has been a lack of therapeutic advance in managing AHF patients, and pharmacological interventions remain limited. Although currently available AHF therapy results in symptomatic relief within the first few days of admission, such improvement is often incomplete with persistent or recurrent symptoms of HF during hospitalization and post-discharge, contributing to in-hospital WHF events, high hospital readmission rates and increased mortality risk. Overall, the current standard-of-care for AHF management include: oxygen in case of hypoxemia, loop diuretics, vasodilators, inotropes, and opiates, with diuretics as the cornerstone of AHF therapy. None of these AHF interventions have been shown in randomized clinical trials to safely reduce the rate of in-hospital WHF, prevent end-organ injury and dysfunction, or reduce either short- or long-term mortality.

Accordingly, there is a significant unmet medical need for new, effective and safe therapeutic agents to treat patients with AHF.

1.2 Development of serelaxin

(Section 3)

Relaxin (H2) is a naturally-occurring peptide hormone that has pleiotropic effects within the cardiovascular system. The activity of the human relaxin-2 peptide hormone is initiated by binding to its cognate G-protein coupled receptors: relaxin family peptide receptors 1 and 2 (RXFP1 and RXFP2). RXFP1 is the specific, high-affinity relaxin-2 receptor whereas RXFP2 binds relaxin-2 with lower affinity. These receptors are abundantly expressed in the cardiovascular (CV), renal and reproductive systems (Hsu et al 2002, Dschietzig et al 2011). Upon receptor binding, relaxin primarily stimulates both the rapid and sustained nitric oxide (NO)-mediated vasodilation pathways. Within minutes of relaxin administration, vasodilation occurs via rapid phosphorylation of nitric oxide synthase (NOS) and production of NO. NO is released from the endothelial cells and acts on adjacent smooth muscle cells to cause rapid relaxation. Within hours of relaxin administration, a sustained NO-mediated vasodilation is also induced via increased endothelial endothelin (ET) type B receptors. Other potential mediators of relaxin's physiological activities include direct induction of atrial natriuretic peptide, and locally produced matrix metalloproteinases.

Relaxin's most widely recognized role is in mediating physiological adaptations in women in response to pregnancy. Endogenous relaxin circulates at levels of 0.1 ng/ml in non-pregnant females during the luteal phase of the menstrual cycle and rises to around 1 ng/ml during the first trimester in pregnant women. There are reports of elevated levels of relaxin in hypertension (Gedikli et al 2009) and congestive HF (Dschietzig et al 2001).

Serelaxin (RLX030) is identical in structure to the naturally-occurring relaxin (H2). Serelaxin administration in non-clinical models leads to decreased systemic vascular resistance (SVR) and increased arterial compliance (AC), contributing to reduction of afterload. Serelaxin also improves renal function by increasing glomerular filtration rate (GFR) and renal blood flow (RBF), as well as causing a moderate increase in natriuresis with a potential effect on preload. In both the systemic and renal vasculature, serelaxin antagonizes the vasoconstrictive effects of angiotensin II and endothelin. Non-clinical studies also suggest that serelaxin may possess connective tissue remodeling, anti-ischemic, anti-apoptotic, and anti-inflammatory activities.

On the basis of nonclinical data, a pilot study in patients with compensated HF was conducted to assess the effect of serelaxin infusion on central hemodynamics and renal safety in HF patients (Study RLX.CHF.001, published as Dschietzig et al 2009). Positive results from this trial led to the development of serelaxin for the treatment of AHF including the Phase II/III RELAX-AHF clinical program, along with a number of hemodynamic profiling studies.

1.3 Overview of nonclinical toxicology

(Section 4)

Serelaxin is pharmacologically-active in a variety of animal species. Toxicology studies included acute and multiple dose general toxicology studies up to 6 months in duration, and reproductive and developmental studies.

The No Observed Adverse Effect Levels (NOAELs) in rats and monkeys after IV administration of serelaxin corresponded to human equivalent doses (based on body surface area) that were 16- and 53-fold higher, respectively, than the human dose evaluated in pivotal clinical studies (approximately 30 µg serelaxin/kg patient body weight per day). The NOAELs were the highest doses evaluated.

Serelaxin was well tolerated in toxicology studies with evidence of pharmacologic activity, and no issues of toxicological significance were identified that would preclude continued clinical development or approval of serelaxin for treatment of AHF.

In cardiovascular safety pharmacology studies, treatment with serelaxin did not result in any adverse effects on heart rate, blood pressure, or ECG intervals at doses up to 100 times the clinical dose of 30 µg/kg/day and did not show any biologically-significant inhibition of cardiac ion channels (hERG (IKr), Na, and Ca) *in vitro*. Developmental and reproductive toxicity data of exogenous relaxin in Rhesus monkeys support short-term serelaxin use in AHF patients.

Following IV administration, serelaxin distributes to blood and highly-perfused organs, including kidneys and liver, and is cleared rapidly from serum with a tri-exponential decay over time in all animal species studied.

Serelaxin is identical to the human relaxin H2 sequence. Relaxin sequence is highly variable across species and these sequence differences probably contribute to the fact that serelaxin is immunogenic in animals. The incidence of anti-serelaxin antibody formation was high in the toxicology studies, and these antibodies sometimes neutralized the pharmacology of serelaxin in an *in vitro* bioactivity assay. Even in the presence of these antibodies, there was still evidence of sustained pharmacodynamic activity in vivo.

In summary, serelaxin was well-tolerated in safety pharmacology studies, general toxicology studies, and developmental and reproductive toxicology studies.

1.4 Overview of human pharmacokinetics

(Section 5)

The pharmacokinetics (PK) of serelaxin were characterized in healthy subjects and in the relevant patient populations following the intended route of administration (IV bolus injection or IV infusion for 20-48 hours). Following a single 10 µg/kg IV bolus injection, serelaxin exhibited tri-exponential decay with the first two phases accounting for 85% of the area under the serum concentration-time curve (AUC). Clearance (CL) was rapid with a mean residence time of 1.6 hours. Following continuous IV infusion, serum concentration of serelaxin increased rapidly within the first few hours and then approached the steady state concentration (Css) starting as early as 4 to 6 hours. Upon cessation of infusion, the concentration declined rapidly following a similar pattern as the IV bolus PK profile.

Serelaxin generally exhibited linear and dose-independent PK across the range of IV doses studied. Serelaxin has shown comparable steady state exposure and systemic clearance following IV administration in healthy volunteers and HF patients. The PK of serelaxin was unaffected by hepatic impairment, and mildly or moderately-impaired renal function did not significantly affect serelaxin systemic CL in patients with AHF. Gender, age (>65 y), race or ethnicity, and body weight also did not appear to have a significant effect on the CL of serelaxin.

No formal drug-drug interaction (DDI) studies were conducted given the low risk of DDI based on a mechanism-driven risk-based analysis. Concomitant medications in patients with AHF did not alter the Css or the CL of serelaxin based on subgroup analyses of Pre-RELAX and RELAX-AHF studies. There was no clinical indication of adverse events (AEs) related to DDI during these studies. As a therapeutic protein, serelaxin is expected to be cleared in the body via catabolism by proteases/peptidases which are not affected by cytochrome P450 enzymes. No statistically significant changes were observed in the levels of pro-inflammatory cytokines in the Phase III RELAX-AHF study, including interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), supporting the concept that no cytokine-mediated therapeutic protein-drug interactions are expected.

With regards to pharmacodynamic (PD) DDIs, the efficacy observed in the RELAX-AHF study was not affected by standard of care medications including ACEi, ARBs, BBs, MRA and IV nitrates. Conversely, the potential of serelaxin to affect the PD of other commonly-used drugs is low, because of the short infusion period.

1.5 Overview of hemodynamics

(Section 6)

Systemic hemodynamic effects of serelaxin were initially explored in a pilot, open-label, safety and PD study (RLX.CHF.001) and then further investigated in a multi-center, double-blind, placebo-controlled study (CRLX030A2201). Renal hemodynamic effects were also investigated in study CRLX030A2202.

The initial pilot study conducted in stable CHF patients showed favorable hemodynamic changes following serelaxin treatment, including reductions in pulmonary capillary wedge pressure (PCWP) and systemic vascular resistance (SVR), and trend to increase in cardiac index/cardiac output (CI/CO), along with improvements in renal function parameters.

In study CRLX030A2201 conducted in AHF patients, 20-hour IV infusion of serelaxin at 30 µg/kg/day led to a significant reduction in PCWP, mean pulmonary arterial pressure (PAP), SVR and pulmonary vascular resistance (PVR), compared to the placebo group. A similar increase in CI/CO from baseline was observed in both serelaxin and placebo-treated patients. There was a significant increase in creatinine clearance over the infusion period in serelaxin vs. placebo patients. Overall, serelaxin was well-tolerated by AHF patients in this study.

Renal hemodynamics were evaluated in congestive heart failure (CHF) patients in CRLX030A2202. In this study, IV infusion of serelaxin at 30 µg/kg/day produced a significant increase in renal plasma flow (RPF) compared to placebo during and following the 24-hour infusion period. Changes in GFR were similar in both treatment groups, while the filtration fraction was decreased by serelaxin relative to placebo. Overall serelaxin was well-tolerated by CHF patients in this study.

In summary, serelaxin has demonstrated favorable hemodynamic effects, i.e., reduction of pre- and afterload resulting in decreased cardiac workload, which provides mechanistic support for the observed improvement in signs and symptoms of congestion reported in RELAX-AHF study. Additionally, serelaxin has shown beneficial effects on renal function.

1.6 Overview of clinical efficacy

(Section 7)

The serelaxin clinical program included approximately 1,400 AHF patients in a Phase II dose-ranging study (Pre-RELAX-AHF) and a Phase III pivotal study (RELAX-AHF). Both studies randomized patients who presented to hospital with sustained dyspnea that persisted after treatment with IV furosemide ≥40 mg, and all patients had normal to elevated blood pressure, increased BNP and/or NT-pro-BNP, and mild to moderate renal dysfunction. Patients were randomized within 16 hours from presentation.

The studies were designed to evaluate the effect of serelaxin on both the short-term and long-term clinically-important endpoints. These included patient reported outcome (PRO)-based assessment of dyspnea, in-hospital WHF, and post-discharge clinical outcomes such as composite of CV death and rehospitalization due to HF or renal failure (RF) through Day 60, days alive out of hospital (DAOOH) through Day 60, and CV mortality through Day 180 (see description of methodology in Section 7.3.1).

1.6.1 Pre-RELAX-AHF

(Section 7.2)

Study Pre-RELAX-AHF was a multicenter, randomized, double-blind, placebo-controlled Phase II study in patients hospitalized with AHF. The study was designed to evaluate the effects of serelaxin on symptom relief, renal improvement (or prevention of impairment), and a range of clinical safety and efficacy endpoints including:

1. Change in dyspnea assessed by AUC of the change from baseline through Day 5 on Visual Analog Scale (VAS)
2. Dyspnea improvement assessed by the proportion of patients with moderate/marked improvement at 6, 12, and 24 h on Likert scale
3. WHF through Day 5
4. Length of hospital stay
5. Persistent renal impairment (creatinine increase of 0.3 mg/dL or more at Day 5 and Day 14)
6. DAOOH through Day 60
7. Composite of CV death or rehospitalization due to HF or RF through Day 60
8. CV mortality through Day 180

A total of 234 patients were randomized in this study, including 229 patients (97.9%) in the modified ITT (mITT) population who received continuous IV infusion for up to 48 hours of placebo or serelaxin (10 μ g/kg/day, 30 μ g/kg/day, 100 μ g/kg/day and 250 μ g/kg/day). Patients in all treatment groups also received background therapy for AHF. The study was designed to evaluate the effects of different doses on a range of clinical variables, but it was not powered to detect statistical differences between the individual serelaxin doses.

Serelaxin at doses ≥30 µg/kg/day produced an improvement in a range of short and long-term clinical variables. Serelaxin treatment improved dyspnea, as assessed by VAS AUC and Likert enpoints. The cumulative incidence of WHF through Day 5 was numerically lower in patients treated with serelaxin (at doses of ≥30 μ g/kg/day)compared to placebo, indicating a potential benefit in the improvement of this important in-hospital outcome in AHF patients. In addition, the probability of cardiovascular (CV) death by Day 180 was also lower for all serelaxin groups in the Pre-RELAX-AHF study, with significantly fewer deaths in the 30 µg/kg/day group than in the placebo group. Serelaxin doses greater than 30 µg/kg/day were not associated with a greater treatment effect.

Serelaxin was generally well-tolerated, with the majority of patients completing the study. There was some evidence of worsening renal function in the 250 µg/kg/day serelaxin treatment group.

The efficacy and safety results summarized above indicated a favorable benefit/risk profile for those patients treated with serelaxin at a dose of 30 µg/kg/day. Therefore, a dosing regimen of 48-hour IV infusion of serelaxin at 30 µg/kg/day was selected for testing in the Phase III pivotal efficacy and safety study, RELAX-AHF.

1.6.2 RELAX-AHF

(Section 7.3)

RELAX-AHF was a randomized, double-blind, placebo-controlled, Phase III pivotal study in patients with AHF receiving standard-of-care treatment.

1.6.2.1 Study Design

Primary efficacy endpoints The primary efficacy assessment of patient-reported change in dyspnea was performed using the VAS and Likert scales in the RELAX-AHF study. The endpoint of VAS AUC assessed both improvement and worsening of dyspnea over 5 days, whereas the Likert endpoint captured only moderate or marked dyspnea improvement for the first 24 hours.

The mean time period from initial patient presentation to time of randomization was approximately 8 hours. VAS was assessed in randomized patients at baseline, 6, 12 and 24 hours, and daily through Day 5 followed by a final assessment at Day 14. The Likert scale was also assessed at the same fixed timepoints relative to the start of study drug infusion. Per protocol, the study was considered to have met its primary objective of demonstrating efficacy of serelaxin in dyspnea relief if either primary endpoint was statistically-significant at the two-sided 0.025 level (alpha was evenly split), or if both tests were significant at the two-sided 0.05 level (Hochberg approach).

In-hospital WHF occurs in 10-30% of hospitalized AHF patients, and it was prospectively incorporated as an **integral component** of the VAS primary efficacy endpoint in the RELAX-AHF program. An in-hospital WHF event was defined in RELAX-AHF as worsening signs and/or symptoms of HF that required an intensification of IV HF therapy (diuretics, vasodilators and/or inotropes) or mechanical ventilatory or circulatory support. By definition, these patients are in significant distress and are experiencing a clinically-significant deterioration requiring immediate rescue therapy. WHF events represent **treatment failures**. In addition, symptom scores reported by the patient following a WHF episode are distorted by the effect of rescue therapy. Hence, for the assessment of dyspnea relief, it was pre-specified in the study protocol that the reported worst dyspnea score for any patient in the study would be assigned after the time of WHF onset. This pre-specified dyspnea score assignment is consistent with the fact that the WHF event, regardless of type of IV rescue therapies received, defines a patient's deteriorating clinical status with a protracted in-hospital clinical course and is associated with an increased risk of mortality as compared to patients without a WHF event. In RELAX-AHF, the worst VAS score reported by any patient was "0". This score was therefore applied to all patients from the point of occurrence of a WHF episode and onwards. Importantly, similar methods of analyzing WHF events have been used in other AHF studies where either the worst rank or score has been assigned to WHF (see Section 7.1.2.2).

Secondary efficacy and other study endpoints The two secondary efficacy endpoints for the study were (1) the composite of CV death or re-hospitalization due to HF or RF through Day 60, and (2) DAOOH through Day 60.

In addition, the WHF endpoint was defined as a composite of in-hospital WHF event, HF rehospitalization, or death up to Day 5 or 14, respectively.

Other pre-specified in-hospital efficacy endpoints included physician's assessment of the signs and symptoms of congestion, doses of IV loop diuretics, and length of hospital stay including days in intensive care unit (ICU)/coronary care unit (CCU).

The study also included an assessment of CV mortality through Day 180 as an additional pre-specified efficacy endpoint, and all-cause mortality through Day 180 as a pre-specified safety endpoint.

Finally, three key biomarkers were assessed: high-sensitivity cardiac troponin T (hs-cTnT) as an indicator of myocardial injury; cystatin-C as a marker of kidney function; and NT-proBNP as a marker of cardiac wall stress.

A total of 1,161 patients were randomized to receive either serelaxin (581 patients) or placebo (580 patients). Of these, 1,035 patients (89.1%) completed the study as planned after receiving serelaxin (530 patients, 91.2%) or placebo (505 patients, 87.1%). The remaining 126 patients (10.9%) who did not complete Day 180, died (106 patients, 9.1%), withdrew consent from follow-up (18 patients, 1.6%), or were lost to follow-up (2 patients, 0.2%). The proportions of patients who either completed the study or withdrew consent were similar for the serelaxin and placebo treatment groups. Vital status at 180 days could not be ascertained in 14 patients (12 patients withdrew from follow-up and 2 were lost to follow-up; 1.2%) with equal distribution between the serelaxin (n=7) and placebo (n=7) groups.

1.6.2.2 Results

Dyspnea relief RELAX-AHF met the primary study objective: treatment with serelaxin resulted in a statistically-significant increase (19.4%) in the VAS AUC Day 0-5 over the observed placebo response (mean difference in VAS AUC = 447.7 mm-hours, p=0.0075). Higher VAS scores compared to placebo were observed at each time point up to Day 5.

The other pre-specified primary efficacy Likert endpoint (moderate or marked improvement at all three time points of 6, 12 and 24 hours) was not different between the serelaxin (26.9%) and placebo-treated (25.9%) patients (p=0.702). Serelaxin treatment was numerically superior to placebo at each individual time point of 6, 12, and 24 hours.

The differences in the reported primary endpoint outcomes generated from the Likert scale vs. VAS mainly reflect the fact that the Likert primary endpoint captured only moderate or marked improvement but not worsening of dyspnea during the first 24 hours. In a post-hoc analysis, when both improvement and worsening of dyspnea were analyzed over 5 days from treatment initiation using the Likert scale, the results were similar to those generated using the VAS scale.

Dyspnea relief achieved through reduction in WHF events Serelaxin treatment produced nearly 50% reduction in the risk of in-hospital WHF event or death through Day 5 with Kaplan-Meier estimates of 6.7% vs. 12.2% for serelaxin and placebo groups, respectively (HR=0.53; 95% CI 0.36 to 0.79, p=0.0016 Wald test from Cox regression model). This reduction was mainly attributed to a decrease in in-hospital WHF events. Fewer serelaxin patients experienced recurrent WHF events through Day 5.

Since in-hospital WHF events were incorporated as an integral component of the primary endpoint, the VAS AUC Day 0-5 was analyzed using both the actual reported vs. assigned scores. The analysis demonstrated that the observed increase in VAS AUC for serelaxin compared to placebo was primarily driven by the reduction of in-hospital WHF events in the serelaxin group.

WHF events were associated with worse in-hospital and longer term outcomes. WHF patients through Day 5 had a significantly prolonged length of initial hospital stay (difference of 8.0 days, p<0.00001) including ICU/CCU stay (difference of 4.9 days, p<0.00001). There was also an increased risk of death at Day 180 for patients experiencing a WHF event (HR=1.98, 95% CI: 1.14 to 3.43, p=0.01479), when compared to patients without WHF events.

A number of pre-specified and post-hoc sensitivity analyses were performed on the primary VAS AUC endpoint including assigning scores other than the pre-specified worst score (VAS=0) to WHF events or the subset of WHF events managed with intensification of IV diuretics only. These analyses consistently favored serelaxin over placebo, irrespective of the assigned dyspnea scores after the WHF event. The consistent results underscore the robustness of findings for treatment benefit of serelaxin.

Secondary endpoints Serelaxin did not improve the secondary efficacy endpoint of the composite of CV death or re-hospitalization due to HF or RF through Day 60. Analysis of the individual component of CV death through Day 60 showed a numerical difference in favor of serelaxin, while the component of re-hospitalization due to HF or RF through Day 60 was numerically in favor of placebo. The observed numerical differences in re-hospitalizations may be partially explained by competing risk: the larger number of patient deaths in the placebo group removed sicker patients (those who were at higher risk for hospitalization) from the study in this group before they could be re-hospitalized. Of note, more patients in the serelaxin arm had a medical history of HF hospitalization in the past year (serelaxin: 37.2% vs. placebo: 31.2%, p=0.0354).

The other secondary efficacy endpoint of DAOOH through Day 60 was numerically in favor of serelaxin, although statistical significance was not achieved.

Worsening heart failure endpoint through Day 14 The earlier benefit in reducing WHF endpoint was maintained through Day 14 (11.4% vs. 15.7%) (HR 0.70, 95% CI: 0.51 to 0.96, p=0.024).

HF signs and symptoms, IV diuretic use, and length of stay Serelaxin treatment was associated with a number of short-term, in-hospital benefits and favorable changes in biomarkers including:

1. Clinically significant improvement in physician-assessed signs and symptoms of congestion, e.g., dyspnea on exertion, orthopnea, edema and rales.
2. Significantly lower doses (furosemide-equivalent) of IV loop diuretics through Day 5: 161.3 mg in serelaxin patients vs. 213.0 mg in placebo patients (p=0.0057); serelaxin patients also transitioned off IV diuretics earlier compared to placebo-treated patients. In addition, serelaxin treatment was associated with less use of inotropic and vasoactive therapies.
3. Significantly shorter length of stay for index hospitalization (mean difference: 0.9 days, p=0.039) and ICU/CCU stay (mean difference: 0.3 days, p=0.029).
4. Beneficial changes in both cardiac (hs-cTnT) and renal (cystatin-C) biomarkers, suggestive of less myocardial injury and renal dysfunction during the most vulnerable early period of exacerbation of AHF. The findings of positive changes in biomarkers also provide a plausible mechanism for a potential long-term mortality benefit.

Cardiovascular mortality For patients treated with serelaxin, a significant 37% relative risk reduction in CV mortality was observed through Day 180 (6.1% vs. 9.6%) (HR=0.63, 95% CI: 0.41 to 0.96, p=0.028). This result is consistent with the reduction in CV mortality reported at this same dose in the Phase II Pre-RELAX-AHF study.

1.7 Overview of safety

(Section 8)

Safety was assessed by analyzing non-serious and serious AEs including deaths, events of interest, physical examination, vital signs, ECGs, laboratory parameters and other markers of HF as collected in the serelaxin and placebo treatment groups. The AE profile after study drug exposure was generally similar in both the serelaxin and placebo groups. Treatment-emergent AEs, i.e., non-serious AEs through Day 5, and SAEs through Day 14, were reported for a total of 53.7% patients treated with serelaxin and 56.1% of patients on placebo. A total of 4.6% of patients on serelaxin and 3.9% on placebo discontinued study drug due to an AE. SAEs and SAEs leading to discontinuation through Day 14 occurred in 15.1% and 0.9% of those receiving serelaxin, and in 13.7% and 0.5% of patients receiving placebo, respectively.

A total of 29.4% of patients treated with serelaxin experienced a 'confirmed blood pressure decrease event' (CBPDE), defined as confirmed SBP decrease by >40 mmHg from baseline and/or to SBP <100 mmHg at any time during study drug infusion. This compares to 18.1% of patients on placebo. Among these patients experiencing a CBPDE, 35.3% of patients on serelaxin completed the infusion receiving a 50% reduced dose (placebo: 30.1%), 9.6% had their dose reduced by 50% but were finally discontinued (placebo: 11.7%), and 54.5% had infusion immediately discontinued while developing CBPDE with SBP <100 mmHg (placebo: 57.3%). The majority of CBPDEs occurred within the first 24 hours and were manageable, leading to an overall low incidence of the AE "Hypotension" through Day 5 (serelaxin: 2.6% vs. placebo: 1.6%).

Minor decreases in hemoglobin (maximum change: -0.3 mg/dL), hematocrit (-1%) and red blood cell (RBC) (-0.04 $x10^{12}$/L) were observed in the serelaxin group versus placebo. These transient decreases are not considered clinically relevant in the AHF setting.

Overall, the AE analysis of the organ systems of heart, kidney and liver showed a favorable safety profile for serelaxin:

- Fewer patients treated with serelaxin compared to placebo experienced AEs indicative of cardiac failure: Standardized MedDRA Query (SMQs) 'Cardiac failure' 8.6% vs. 11.6%) and AEs indicative of cardiac arrhythmias (SMQs 'Cardiac arrhythmias'/ 'Torsade de pointes/QT prolongation': 6.2% vs. 8.4%) through Day 14.
- AEs associated with renal impairment (SMQ 'Acute renal failure') were reported by a smaller percentage of patients in the serelaxin group (5.6%) compared to the placebo group (8.9%) through Day 14. There were also fewer patients in the serelaxin arm (9.6%) who experienced an increase of serum creatinine >0.5 mg/dL at Day 5 compared to placebo (15.6%) (p=0.003).
- Hepatic AEs (SMQ 'Drug related hepatic disorders - comprehensive search') were reported by fewer patients in the serelaxin group (0.9%) compared to the placebo group (2.8%).

Across all HF studies conducted with serelaxin, there were only sporadic reports of AEs that might have been indicative of immune system disorders or hypersensitivity/allergy. The majority of these AEs were of mild severity and not considered medically-significant; no imbalance between serelaxin and placebo has been observed. One out of 559 patients tested in HF studies was anti-serelaxin antibody positive on Day 30 post-dose (study CRLX030A2201). In this patient, the antibody titer was too low to be determined, and no neutralizing activity was detected. In addition, no AE indicating a hypersensitivity reaction was reported.

The all-cause mortality through Day 180 was a pre-defined safety endpoint in the Phase III RELAX-AHF study. In the serelaxin group, 42 patients died (Kaplan Meier estimate: 7.3%) compared to 65 patients (Kaplan Meier estimate: 11.3%) in the placebo group (p=0.020, ITT set) (HR=0.63, 95% CI: 0.43 to 0.93). A similar result was observed in the safety population showing 41 deaths with serelaxin (7.2%) and 64 with placebo (11.2%) (HR=0.63, p<0.02). The upper boundary of the 95% confidence interval for the HR of all-cause mortality at Day 180 was <1.0 demonstrating the safety of serelaxin on this important outcome measure and providing persuasive evidence for no harm.

Overall, the data demonstrated that serelaxin was generally safe and well-tolerated when administered to AHF patients.

1.8 Regulatory basis for approval

Novartis submitted a BLA based on clinical evidence from the Pre-RELAX-AHF and RELAX-AHF studies which provide compelling support for a well-tolerated new therapeutic agent for the treatment of AHF. The data presented in the serelaxin BLA satisfy the requirements for a single pivotal trial submission as outlined in the Food and Drug Administration Modernization Act of 1997.

In 1962, Congress amended the Federal Food, Drug, and Cosmetic Act to add a requirement that, to obtain marketing approval for a new drug, manufacturers must demonstrate the effectiveness of their products through the conduct of adequate and well controlled investigations ("substantial evidence"). Subsequently, in the Food and Drug Administration Modernization Act of 1997, Congress clarified that the FDA may consider "data from one adequate and well-controlled clinical investigation and confirmatory evidence" to constitute "substantial evidence" if FDA determines that such data and evidence are sufficient to establish effectiveness (e.g. a single study with very convincing statistical results and internal consistency with supportive evidence). In addition to considerations of efficacy, sufficient data / exposure to enable adequate characterization of the safety profile and potenial risks of the proposed indication and population are also needed.

Overall, for serelaxin, evidence is provided showing its benefits on a number of clinically important endpoints and a very benign safety profile. Importantly, the RELAX-AHF trial met its primary endpoint of AUC VAS to Day 5 by showing a statistically significant and clinically relevant 19.4% improvement on serelaxin. This finding was mainly driven by an almost 50% reduction of in-hospital WHF. These results are supported by significant benefits on a number of additional clinically meaningful and biologically relevant endpoints, including reduction of length of hospital stay and Day 180 CV and all-cause mortality. Furthermore, the results of Pre-RELAX-AHF are consistent with those obtained in the RELAX-AHF trial. Therefore, the current application provides a robust body of evidence establishing a positive benefit risk ratio for serelaxin in the treatment of acute heart failure.

1.9 Benefit/Risk

(Section 9)

AHF represents a life-threatening disease with poor prognosis for patients and an enormous burden on health care systems. Although current standard-of-care is available to relieve symptoms of dyspnea and congestion in AHF, the disease burden remains high, and no currently-approved therapy has been shown to safely reduce in-hospital WHF events.

In the RELAX-AHF program:

- Serelaxin infused for 48 hours in RELAX-AHF resulted in a clinically and statistically significant 19.4% improvement in VAS AUC score for dyspnea from baseline through Day 5 compared to placebo. This was largely driven by a reduction in physician-assessed in-hospital WHF events. The effect of serelaxin in reducing WHF was highly statistically significant through Day 5 (HR=0.53).
- Serelaxin-treated patients experienced greater improvements in signs of congestion, while receiving lower doses and a faster transition off of IV loop diuretics, and less use of inotropic/vasoactive therapies.
- IV infusion of serelaxin produced a rapid and sustained reduction in PCWP and PAP in hospitalized AHF patients (study CRLX030A2201). These findings provided mechanistic evidence supporting the improvement in patient-reported dyspnea and other symptoms or signs of congestion.
- Serelaxin was associated with a significant reduction in the length of stay for the index hospitalization including the duration of ICU/CCU stay.

- A 37% relative reduction in both CV and all-cause mortality at Day 180 was observed in the serelaxin-treated patients in RELAX-AHF. A similar finding of reduced mortality was also reported in serelaxin-treated patients in the Phase II Pre-RELAX-AHF study.
- Favorable changes were seen in cardiac and renal biomarkers (hs-cTnT, cystatin-C, etc.) among serelaxin patients.
- Serelaxin administered as a 48-hour IV infusion was well-tolerated with an overall safety profile similar to placebo except for an increase in hypotension-related AEs. BP decreases can occur in some patients due to the vascular mechanism of action of the drug, but these are manageable with regular BP monitoring, and dose reduction or discontinuation of serelaxin infusion, as applied in RELAX-AHF. These BP monitoring measures are also proposed for inclusion in the prescribing information.

In conclusion, results of the RELAX-AHF, Pre-RELAX-AHF and other studies provide compelling support for serelaxin as a safe therapeutic agent that improves the symptoms of AHF through reduction of the rate of in-hospital WHF events. The clinical program demonstrated an improvement in physician-assessed HF signs and symptoms, lower use and faster transition off IV diuretics, and shorter length of hospital stay. In addition, IV infusion of serelaxin produced rapid and sustained reduction in PCWP and PAP in hospitalized AHF patients. These results collectively demonstrate a positive and clinically-meaningful impact of serelaxin on the in-hospital course of AHF patients.

A significant reduction in CV and all-cause mortality was observed following serelaxin treatment providing, at a minimum, persuasive evidence of no harm. Confirmation of the potential mortality benefit observed in the Pre-RELAX-AHF and RELAX-AHF studies is being sought in an ongoing study (RELAX-AHF-2).

2 Acute Heart Failure

2.1 Epidemiology

AHF is a major growing public health problem in the US, with AHF hospitalizations tripling since 1979 and reaching >1 million hospitalizations annually. It is now the most common cause of hospitalizations among people older than 65 years in the US (Fang et al 2008; Go et al 2013; Roger 2013; Yancy et al 2013). AHF also represents a major economic burden to the US Health Care System with a total of estimated direct and indirect costs of $34 billion in 2010 (Heidenreich et al 2011). The lifetime risk of developing HF is 20% for Americans ≥40 years of age, and approximately 5.1 million persons in US have clinically defined HF (Yancy et al 2013). The prevalence of HF in the US general population is expected to increase due to aging of the population, the persistent incidence of newly-diagnosed HF, improvement in the treatment of acute conditions such as acute coronary syndrome, and improved survival of patients with chronic heart failure (CHF) (Roger 2013; Yancy et al 2013). According to a recent projection by the American Heart Association, the prevalence of HF is expected to increase by 25% in 2030, with a corresponding increase in the associated costs up to $95 billion in 2030 (Heidenreich et al 2011).

Moreover, in addition to costs, hospitalization for AHF represents a sentinel prognostic event in the clinical course of many HF patients, which is associated with ~4% in-hospital mortality, ~30% readmission rate at 3-6 months, and 20-35% mortality at 1 year after hospitalization (Adams et al 2005; Curtis et al 2009; Fonarow et al 2007; Heidenreich and Fonarow 2006; Levy et al 2002; Loehr et al 2008). The 30-day post-admission mortality rates decreased from 12.6% to 10.8% from 1993 to 2006; however, this was due to lower in-hospital death rates, whereas the post-discharge mortality actually increased from 4.3% to 6.4% during the same time frame (Bueno et al 2010). The reported 5-year relative survival is approximately 62% (age and sex adjusted) for HF, compared to the 50% and 50-57% survival rate reported for stroke and all cancer entities, respectively (Askoxylakis et al 2010).

AHF remains a significant unmet medical need considering its increasing prevalence and poor clinical prognosis.

2.2 Pathophysiology

AHF is defined as the rapid onset of, or change in, HF signs and symptoms (e.g., breathlessness, orthopnea, paroxysmal nocturnal dyspnea and fatigue) that require urgent therapy (McMurray et al 2012). Recent registries have consistently shown that more than 80% of AHF patients present with dyspnea, 60-80% with renal impairment, and the majority also have normal to elevated systolic BP (Adams et al 2005; Gheorghiade et al 2006a; Gheorghiade et al 2006b; Gheorghiade and Pang 2009). Although symptomatic relief does occur in many patients within a few days of admission, such improvement is often incomplete with persistent or recurrent HF symptoms during hospitalization and post-discharge, and re-hospitalizations are common. For example, 60% of patients in the IMPACT-HF trial were discharged with continuing symptoms of dyspnea (or fatigue); after 60 days, 45% still experienced symptoms of WHF and as many as 25% of patients required rehospitalization (Gheorghiade et al 2006b).

An episode of AHF and its long-term sequelae has been proposed to be characterized by two phases (1) short-term central, pulmonary, and peripheral venous congestion coupled with vasoconstriction that contributes to myocardial overload and renal dysfunction that trigger acute exacerbation of HF signs and symptoms; and (2) an increased long-term risk of mortality and morbidity as a result of end-organ damage to the heart or dysfunction of the kidneys during the early critical phase of AHF episodes. With each AHF episode, there may be additional myocardial injury or renal dysfunction that contributes to progressive cardiac/renal remodeling and organ dysfunction, leading to an inevitable downward spiral (Gheorghiade et al 2005).

Elevated biomarkers during the AHF hospitalization that are indicative of myocardial injury (cardiac troponins), renal dysfunction (cystatin-C), and cardiac wall stress (BNP or NT-proBNP) have all been associated with poor clinical outcomes (Bettencourt et al 2004; Damman et al 2007; Gottlieb et al 2002; Maisel et al 2008; Metra et al 2012; O'Connor et al, 2011; Thygesen et al 2012a; Yancy et al 2013). Natriuretic peptides and cardiac troponins have been recommended as valuable biomarkers for risk stratification in AHF patients (Yancy et al 2013).

2.3 Treatment

2.3.1 Goals of treatment

The short-term goals of AHF treatment are:

- Stabilize and improve HF symptoms primarily caused by pulmonary congestion.
- Prevent worsening of the patient's clinical condition and associated in-hospital events, such as WHF (defined as worsening signs and symptoms of HF requiring intensification of rescue therapy), and reduction of the length of hospital stay.
- Prevent early end-organ injury or dysfunction of heart, kidneys and other vital organs.

Long-term goals include:

- Reduce post-discharge events such as rehospitalization and mortality.

WHF events occur in 10-30% of hospitalized patients following initial stabilization (Torre-Amione et al 2009, Cotter et al 2010). It is a distinct clinical event that represents a severe deterioration of AHF signs/symptoms requiring urgent intervention. The occurrence of WHF represents a **treatment failure**. In-hospital WHF is associated with a longer length of hospital stay and an increased risk of subsequent death or rehospitalization (Ander et al 2004; Cotter et al 2010; Gheorghiade et al 2006b; Metra et al 2010; Metra et al 2011). Therefore, the prevention of in-hospital WHF events constitutes an important treatment goal.

2.3.2 Current treatment options

The current standard-of-care for AHF includes: oxygen in case of hypoxemia, loop diuretics, vasodilators, inotropes, and opiates, with IV diuretics as the cornerstone therapy for AHF (class of recommendation: I; level of evidence: B) (Yancy et al 2013). There has been no therapeutic advance in managing AHF patients in the past decade.

Therapies with vasodilative properties include IV nitrates, sodium nitroprusside and nesiritide (Yancy et al 2013). IV nitrates act primarily through venodilation, lowering preload, and may help to reduce pulmonary congestion. However, in a recent review of four randomized, controlled AHF trials comparing nitrates (nitroglycerin and isosorbide dinitrate) with alternative interventions (mainly diuretics), no significant differences were observed between nitrate therapy and alternative interventions with regard to symptom relief and haemodynamic variables (Wakai et al 2013). In addition, there are no data suggesting that IV vasodilators improve outcomes in patients hospitalized with AHF (Yancy et al 2013). Therefore the use of IV vasodilators are recommended as adjuvants to diuretic therapy for the relief of dyspnea in the hospitalized AHF patient with normal or elevated blood pressure (Yancy et al 2013). The inconsistent evidence supporting the use of nitrates in managing AHF (without acute coronary syndrome) has translated into considerable variability in their rate of use.

Overall, these currently recommended pharmacological therapies are generally focused on achieving dyspnea relief and reducing congestion, with little or no evidence of fulfilling other important short-term AHF treatment goals, including reducing in-hospital WHF events. In addition, none of these available interventions have been shown to exert a beneficial impact on mortality in treated AHF patients (McMurray et al 2012; Yancy et al 2013).

2.3.3 Challenges in developing new therapies

No drugs have been approved for the treatment of AHF since nesiritide was approved by the FDA in 2001, based on its short-term improvements in hemodynamic status and patient-reported dyspnea. An array of drugs have been investigated for their treatment potential, but with no solid evidence of clinical benefits and with concerns over safety (Felker et al 2010, Marti et al 2012, McMurray et al 2012). Notably, these include calcium sensitizer levosimendan (Mebazaa et al 2007; Packer et al 2013), A1 adenosine receptor antagonist rolofylline (Massie et al 2010), endothelin type A/B receptor antagonist tezosentan (McMurray et al 2007), and vasopressin V_2 receptor antagonist tolvaptan (Gheorghiade et al 2007; Konstam et al 2007). There are a number of major issues contributing to the challenges in evaluating AHF therapies (Allen et al 2009; Gheorghiade et al 2005):

- Limited understanding of the underlying pathophysiologic processes
- Patient selection may play a role in determining the effects of a given therapy
- Timing of drug administration: Most AHF trials conducted to-date have used interventions initiated days after the initial presentation. The lack of efficacy in prior studies may be related to failure to implement treatment within the first several hours following initial clinical presentation, at a time when patients are most symptomatic and clinically unstable, and are most vulnerable to end-organ injury and dysfunction.
- Differences in clinical study design and in definition of endpoints. In particular, studies designed to evaluate symptomatic improvement in AHF patients should incorporate the occurrence of in-hospital WHF events. These WHF events represent true treatment failure and the subsequent intensification of rescue therapy distorts the evaluation of the originally randomized treatment. Therefore, these events should be appropriately incorporated into the primary endpoint of symptom assessment and/or treatment response (Section 7.1.2).

In summary, AHF is a complex disease entity with substantial morbidity and mortality risks and associated with enormous utilization of health resources and cost. Given the increasing incidence of AHF, its very poor prognosis and the major limitations of current standard-of-care to improve short and long-term outcomes, there is a significant unmet medical need to achieve sustained symptom relief, reduce in-hospital WHF events, mitigate early end-organ injury or dysfunction, and ultimately reduce cardiovascular mortality.

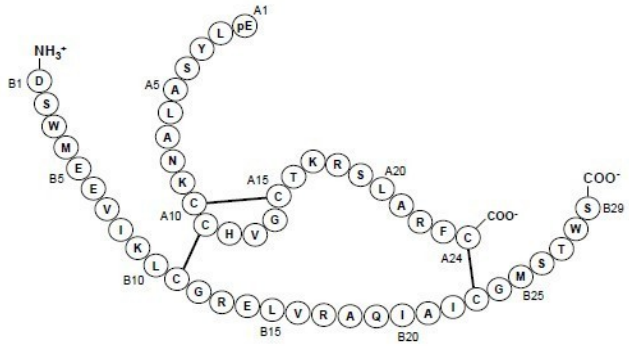
3 Serelaxin Product History

Serelaxin is a recombinant form of human relaxin-2 hormone, which is expressed in *E. coli* and has an identical amino acid sequence and structure to the mature, naturally occurring human relaxin-2 molecule.

3.1 Biology of relaxin

Relaxin is a naturally occurring peptide hormone that was first described in 1926 (Hisaw 1926). It was termed "relaxin" because of a putative role in relaxing pelvic ligaments and softening the pubic symphysis in pregnant guinea pigs (Fevold et al 1930). It is a member of the relaxin peptide family, which in humans consists of relaxin-1, relaxin-2, relaxin-3 and the insulin-like peptides (INSL) -3, 4, 5 and 6. While three human relaxin genes have been identified (RLN1, RLN2, RLN3), the peptide encoded by the RLN2 gene, relaxin (H2), is the circulating form in humans. In nature, it is initially synthesized in the cell as a pre-pro-peptide that is processed to yield the mature relaxin molecule, which is a 2-chain heterodimeric (24-amino acid A chain + 29-amino acid B chain) molecule covalently bonded by 2 disulfide bridges (Figure 3-1). The A-chain has an additional internal disulfide bridge. Mass spectrometric analysis of purified human relaxin indicates a molecular weight of 5963 daltons for mature relaxin.

Figure 3-1 Structure of naturally occurring human relaxin-2 hormone



In men, relaxin is synthesized and is present in the prostate (Colon et al 1994) and very low levels may be present in the circulation (Dschietzig et al 2001; Wolf et al 2013). Little is known of the physiology of relaxin in men, but it may play a role in sperm motility (Ferlin et al 2012).

Relaxin is synthesized in the corpus luteum of the ovary. Women are exposed to monthly elevations of relaxin (~50 pg/mL) during the luteal phase of each menstrual cycle (Stewart et al 1990). Relaxin levels during the 9 months of pregnancy are much higher, with a mean concentration of 1.2 ng/mL (Szlachter et al 1982) and concentrations up to 5.7 ng/mL (Davies et al 2008) have been reported. Relaxin is also normally present in breast milk (Conrad 2010). Relaxin has been associated with many of the adaptive maternal physiological responses to pregnancy. Relaxin is elevated during pregnancy and is believed to contribute to the early maternal hemodynamic adjustments required to adapt to the demands of the growing fetus (Conrad 2011b). A decrease in systemic vascular resistance, rise in cardiac output and arterial compliance and increase in renal blood flow and glomerular filtration rate occur coincident with the rise in relaxin (Bathgate el al 2006). Animal studies show that early hemodynamic changes of pregnancy can be prevented using neutralizing antibodes against relaxin (Conrad 2011a).

3.2 Potential use of relaxin in treatment of AHF

In 2004, Corthera was approached by Dr. Thomas Dschietzig with the proposal to explore the hemodynamic properties of serelaxin in an investigator initiated trial in compensated heart failure patients. The cardiac and renal benefits that are hypothesized to be produced by relaxin during pregnancy suggest that serelaxin may be beneficial to AHF patients.

3.3 Mechanism of action

3.3.1 Relaxin receptors

The activity of the relaxin (H2) peptide hormone is initiated by binding to its cognate G-protein coupled receptors RXFP1 and RXFP2. RXFP1 is the specific, high affinity relaxin receptor whereas RXFP2 binds relaxin (H2) with lower affinity. RXFP2 is the cognate receptor for INSL3 (Kumagai et al 2002; Halls et al 2005). It is believed that in vivo, serelaxin acts only through RXFP1 as knockout mice have a complete absence of vasodilation in response to serelaxin (Debrah et al 2008).

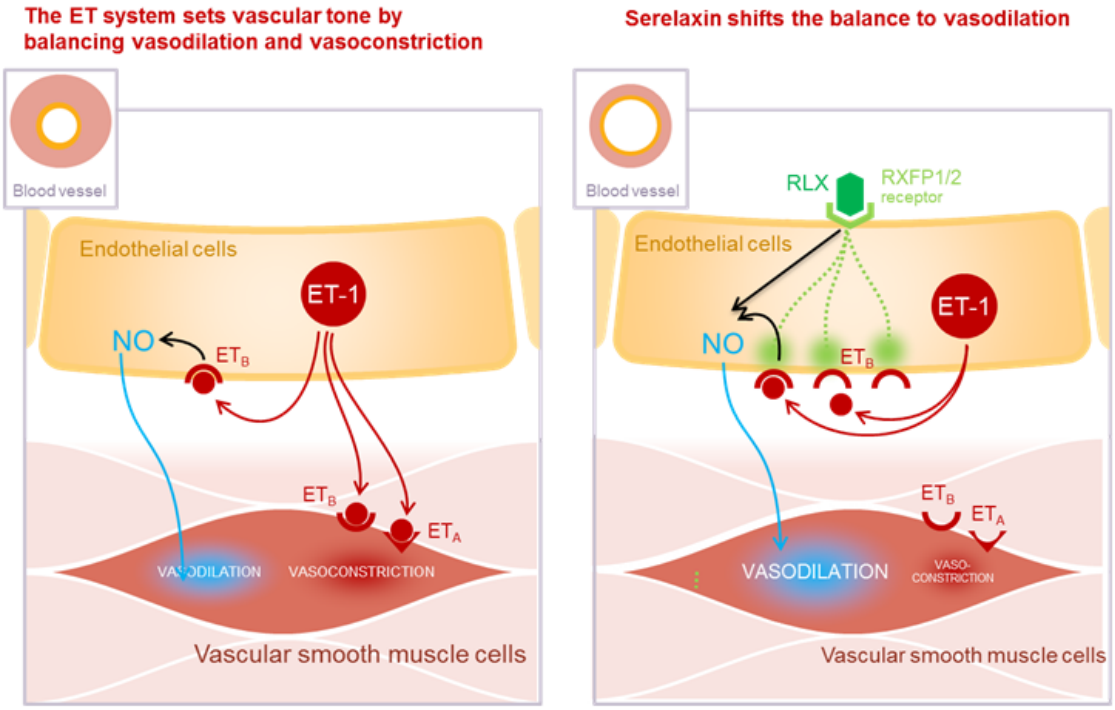
These receptors are abundantly expressed in cardiovascular (CV), renal and reproductive systems, notably on the surfaces of endothelial cells and smooth muscle cells (Hsu et al 2002, Dschietzig et al 2011). A non-clinical study, using immuno-histochemistry (IHC), confirmed that RXFP1 receptors are located in fetal/juvenile and adult human and rat tissues from heart, lung, kidney, liver, brain and spleen. In heart, RXFP1 receptors are localized in cardiomyocytes and smooth muscle cells of tunica media of blood vessels. In lung, the smooth muscle cells of bronchi and tunica media of blood vessels were positively stained. Histological analysis of kidney tissue revealed the presence of RXFP1 receptors in tubular cells of proximal convoluted tubules, mesangial cells in glomeruli and smooth muscle cells of tunica media of blood vessels. Relaxin has also been detected in rat renal mesenteric and thoracic aorta and in human saphenous vein, mammary artery and vessels in the skin (Novak et al 2006).

3.3.2 Signaling pathways underlying hemodynamic responses to relaxin

The primary hemodynamic effect of relaxin is vasodilation, and NO is believed to act as the mediator of relaxin's vasodilatory effect. Relaxin helps regulate changes in NO levels through several different pathways: there are rapid direct effects on NO mediated through phosphorylation of nitric oxide synthase, and less direct mechanisms involving ET_B receptor expression (See Figure 3-2). These combine to give both rapid and sustained responses.

The endothelin system is dysregulated in AHF, and it is characterized with pathologically elevated levels of ET-1 and also alterations in the expression of ET-1 receptors, i.e., increase in ET_A receptor vs. decrease in ET_B receptor density (Asano et al 2002, McMurray et al 1992). These changes result in arterial and venous vasoconstriction that contributes to the hemodynamic changes and associated symptoms in AHF patients (Schneider et al 2007, McMurray et al 1992, Good et al 1994, Masson et al 2006). Mediated through NO and ET_B receptors, serelaxin shifts the vasoconstricted condition to the state of vasodilation (Figure 3-2).

Figure 3-2 Vasodilatory effects of RXFP1 receptor activation



Serelaxin binding to RXFP1 on endothelial cell leads to (1) rapid NOS activation via phosphorylation; (2) sustained NOS activation via endothelial ET_B receptors which also mediate ET-1 clearance.

As shown in Figure 3-2, rapid signaling occurs within minutes following receptor binding in endothelial cells where serelaxin stimulates rapid activation of phosphatidylinositol-3-kinase (PI3K) and NOS (McGuane et al 2011).

Serelaxin also upregulates ET_B receptor gene expression in endothelial and HeLa cells within 2 hrs, and the ET_B receptor density is increased following a 6 hr exposure (Dschietzig and Stangl 2003).

3.3.3 Hemodynamic and renal changes caused by serelaxin

Relaxin dilates systemic and renal blood vessels and increases global arterial compliance, effects that potentially mediate the early maternal hemodynamic and renovascular adjustments to the demands of pregnancy (Conrad 2010, Conrad 2011a, Conrad 2011b, Conrad and Shroff 2011). With these hemodynamic effects, serelaxin may be beneficial to HF patients by not only creating a favorable hemodynamic environment but also by exerting positive effects on the kidneys.

Serelaxin increases renal blood flow by binding to its receptors on the endothelial cells of small renal arteries, which increases ET_B receptor activation leading to vasodilation (Danielson et al 2000). Natriureses and diuresis are stimulated by binding of serelaxin to its receptor on the epithelial cells of proximal tubules and inner medullary collecting duct (Ferreira et al 2009), again via activation of the ET_B receptor, which inhibits Na+ and Cl- transport in several tubular segments and may inhibit vasopressin-induced water resorption by the collecting duct.

3.3.4 Additional potential mechanisms

Other non-clinical studies suggest that serelaxin may possess connective tissue remodeling, anti-ischemic, anti-apoptotic and anti-inflammatory activities (Unemori et al 1993, Unemori et al 1996, Garber et al 2001, Williams et al 2001, Garber et al 2003, Kenyon et al 2003, McDonald et al 2003, Samuel et al 2003a, Samuel et al 2003b, Samuel et al 2004b, Du et al 2010). Serelaxin exerts benefical effects on connective tissue remodeling to ameliorate cardiac hypertrophy and fibrosis (Samuel et al 2011). Prevention of inflammation and oxidative stress may also contribute to cardioprotection (Du 2010). Relaxin has been shown to have anti-fibrotic activity in lung, liver, kidney, skin, heart when administered in vivo (Bennett 2009). Endogenous relaxin may play a role in prevention of age related fibrosis, as relaxin knockout mice show an age-related cardiac and renal fibrosis (Hewitson et al 2012). A similar phenotype is seen in mice that have no relaxin receptors (Kamat et al 2004).

3.4 Product development and regulatory history

An IND was initiated by Corthera in 2007 to evaluate use of serelaxin in the treatment of acute heart failure. The serelaxin clinical program in AHF consists of (1) two randomized clinical studies, i.e., Phase II study Pre-RELAX-AHF and Phase III pivotal study RELAX-AHF, that enrolled similar hospitalized AHF patient population and infused the drug for a similar duration, i.e., continuous 48-hour IV infusion of serelaxin or placebo in addition to the background standard-of-care AHF therapies, and (2) a pilot safety and hemodynamic study RLX.CHF.001 in stable CHF patients, a central hemodynamic study CRLX030A2201 in AHF patients, and a renal hemodynamic study CRLX030A2202 in CHF patients. In addition, three pharmacokinetic (PK) studies were completed in healthy volunteers and in patients with hepatic impairment/normal hepatic function, respectively (R0006g, CRLX030A2103, CRLX030A2101).

All the completed and currently ongoing clinical studies related to the development of serelaxin in AHF indication are summarized in Table 3-1 and Table 3-2.

Table 3-1 Completed serelaxin clinical studies relevant to the AHF program

Study code (Study name)	Study design	Major study Objectives	Study population	Dose regimen (# subjects)
Phase I				
R0006g	Phase I cross-over PK and safety study	PK, immunogenicity and safety	25 healthy female subjects	Single 10 µg/kg IV bolus, followed by a minimum 7-d washout period and then intravaginal serelaxin (1500 µg, n=15) or intracervical serelaxin (750 µg, n=10)
RLX.CHF.001 (Pilot Study in CHF)	Phase I open-label single-center safety and hemodynamic study	To evaluate the hemodynamic effect of IV serelaxin infusion on PCWP, CO/CI	16 patients with stable CHF	24-hour IV infusion, (8-hour intervals) with escalating doses: 10, 30, 100 µg/kg/day (n=4);

Study code (Study name)	Study design	Major study Objectives	Study population	Dose regimen (# subjects)
		and SVR, as well as renal safety (serum creatinine, BUN and uric acid)		250, 480, 960 µg/kg/day (n=6); 960 µg/kg/day (n=6)
CRLX030A2101 (Novartis)	A Phase I single-dose, open-label, parallel group study in subjects with hepatic impairment	To assess the PK of serelaxin in subjects with mild, moderate and severe hepatic impairment, compared to healthy control subjects	25 subjects with mild (n=9), moderate (n=8) & severe (n=8) hepatic impairment vs. 24 matched subjects with normal hepatic function	24-hour IV infusion: Serelaxin 30 µg/kg/day (n=49)
CRLX030A2103	A Phase I, double-blind, placebo-controlled, parallel group, exploratory study in healthy subjects	To evaluate the safety, tolerability, PK/PD of IV infusion of serelaxin at three dose levels in Japanese healthy subjects, with an open-label comparison to Caucasian subjects at one dose level	32 Japanese male and female healthy subjects and 8 Caucasian male and female healthy subjects	48-hour IV infusion: Placebo (n=8) Serelaxin: 10 µg/kg/day (n=8) 30 µg/kg/day (n=16) 100 µg/kg/day (n=8)
Phase II				
RLX.CHF.003.PRE* (Pre-RELAX-AHF)	Phase II multicenter, randomized, double-blind, placebo-controlled safety and efficacy study	Dose-ranging study evaluating effects of IV serelaxin on a number of clinical endpoints, including dyspnea improvement, in-hospital outcomes & CV & all-cause mortality through Day 180. Study supported dose selection for Phase III RELAX-AHF study.	234 randomized patients hospitalized with AHF, normal to elevated blood pressure, and mild to moderate renal impairment	48-hour IV infusion: Placebo (n=62) Serelaxin: 10 µg/kg/day (n=40) 30 µg/kg/day (n=43) 100 µg/kg/day (n=39) 250 µg/kg/day (n=50)
CRLX030A2201	A Phase II multicenter, randomized, double-blind, placebo-controlled, parallel group, hemodynamic study	To evaluate the central hemodynamic responses to IV serelaxin infusion in AHF patients	71 patients hospitalized with AHF	20-hour IV infusion: Placebo (n=37) Serelaxin: 30 µg/kg/day (n=34)
CRLX030A2202	A Phase II multicenter, randomized, double-blind, placebo-	To evaluate the renal hemodynamic responses to IV serelaxin infusion	87 CHF patients with mild to moderate renal impairment	24-hour IV infusion: Placebo (n=48) Serelaxin: 30 µg/kg/day

Study code (Study name)	Study design	Major study Objectives	Study population	Dose regimen (# subjects)
	controlled, parallel group, renal hymodynamic study	in CHF patients		(n=39)
RLX.CHF.002	Phase II multicenter, double-blind, placebo-controlled, hemodynamic and safety study in AHF patients: (Study terminated earlier to initiate RLX.CHF.003. Pre)	To evaluate the safety and central hemodynamic responses to IV serelaxin infusion in AHF patients	11 AHF patients enrolled (n=80 planned)	48-hour IV infusion: Placebo (n=3) Serelaxin: 100 µg/kg/day (n=4) 500 µg/kg/day (n=4)
Phase III				
RLX.CHF.003* (RELAX-AHF)	Phase III multicenter, randomized, double-blind, placebo-controlled efficacy and safety study	Pivotal study to evaluate whether IV serelaxin infusion is superior to placebo in 1) dyspnea improvement and other short-term clinical endpoints; 2) reducing the longer-term (Day 60 and Day 180) mortality/morbidity in these patients; 3) the safety of relaxin administration in AHF patients.	1,161 patients hospitalized with AHF, normal to elevated blood pressure, and mild to moderate renal impairment	48-hour IV infusion: Placebo (n=580) Serelaxin: 30 µg/kg/day (n=581)

CAD: Coronary Artery Disease; CO: cardiac output, ESRD: End Stage Renal Disease;
SVR: Systemic Vascular Resistance; PCWP: Pulmonary Capillary Wedge Pressure

Table 3-2 Ongoing serelaxin clinical studies relevant to the AHF program

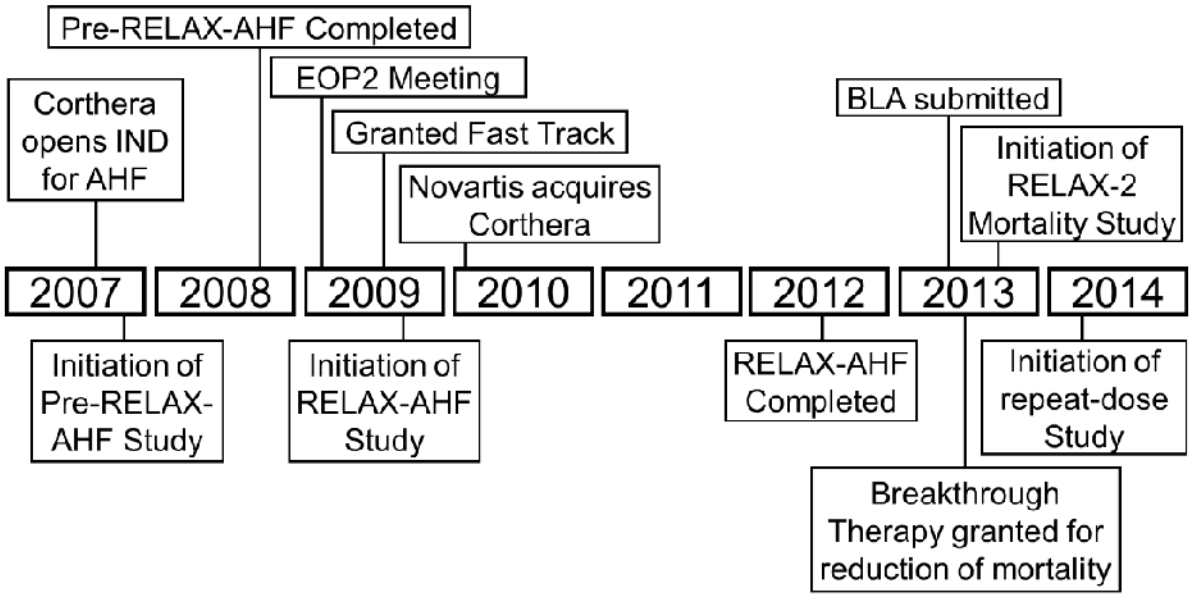
Study code (Study name)	Study design	Major study objectives	Study population	Dose regimen (# subjects)
CRLX030A2301 (RELAX-AHF 2)	A Phase III multicenter, randomized, double-blind, placebo-controlled study	To demonstrate that serelaxin is superior to placebo in reducing CV death in AHF patients during a follow-up period of 180 days	Approximately 6,375 patients hospitalized with AHF, normal to elevated blood pressure, and mild to moderate renal impairment	48-hour IV infusion: Patients randomized 1:1 to placebo vs. serelaxin 30 µg/kg/day
CRLX030A2209	A Phase IIb multicenter, double-blind, placebo-controlled study evaluating the safety of repeat doses of IV serelaxin in CHF patients	To assess the proportion of CHF patients who develop anti-serelaxin antibodies at any time following repeated administration of IV infusion of serelaxin for up to 48 hours	Approximately 300 compensated CHF patients, with a NT-proBNP value >300 pg/mL	48-hour IV infusion at randomization, Weeks 4 and 8: Placebo (n=100) Serelaxin (n=200) 30 µg/kg/day
CRLX030A2102	A Phase I, open-label, parallel group study in patients with severe renal impairment or ESRD on hemodialysis	To assess the PK of serelaxin in patients with severe renal impairment or ESRD on hemodialysis, and compare to matched healthy control subjects	36 subjects with severe renal impairment (n=6), ESRD on hemodialysis (n=12), and matched healthy control subjects (n=18)	4-hour IV infusion at single dose of serelaxin 10 µg/kg

ESRD: End Stage Renal Disease;

Serelaxin was granted Fast Track status in 2009, and in 2013 it was granted Breakthrough Therapy designation for reduction of cardiovascular mortality (Figure 3-3). An outcome study (Study RELAX-AHF-2) is underway to confirm the cardiovascular mortality reduction seen in RELAX-AHF, and another study is underway to evaluate the safety and tolerability of repeat dosing with serelaxin in CHF patients.

Serelaxin was also studied previously for other indications, including cervical ripening, infertility, orthodontic therapy, fibromyalgia and systemic sclerosis. These indications targeted different patient populations, and utilized different routes of administration and durations of treatment. Therefore, results obtained in those studies are not directly pertinent to the proposed use in treatment of AHF patients.

Figure 3-3 Regulatory development of serelaxin



3.5 Product description and proposed indication

Serelaxin is supplied as a sterile, preservative-free solution for infusion (1 mg/mL). One vial of 3.5 mL contains 3.5 mg of serelaxin. The excipients utilized in serelaxin concentrate for solution for infusion are standard pharmacopoeial excipients commonly used in intravenous formulations. The drug product of serelaxin is formulated as sterile acetate buffered solution (20 mM sodium acetate pH 5.0).

The quantity of serelaxin to be administered is calculated based on patient weight.

The current BLA is seeking approval for serelaxin for the following indication :

> "Serelaxin is a recombinant form of relaxin-2 hormone indicated to improve the symptoms of acute heart failure through reduction of the rate of worsening of heart failure."

A review of the key nonclinical and clinical data supporting the serelaxin application is provided in the following sections.

4 Nonclinical Toxicology

Serelaxin is pharmacologically-active in a variety of animal species. Toxicology studies supporting the AHF indication were conducted in rats and monkeys and included acute, multiple dose and continuous infusion studies up to 6 months in duration, and reproductive and developmental studies (Table 4-1 and Table 4-2).

The No Observed Adverse Effect Levels (NOAELs) in rats and monkeys after intravenous administration of serelaxin corresponded to human equivalent doses (based on body surface area) that were 16- and 53-fold higher, respectively, than the human dose evaluated in pivotal clinical studies (30 µg serelaxin/kg patient body weight/day).

Table 4-1 Intravenous and subcutaneous repeated dose toxicity studies of serelaxin

Species	Route of administration	Doses (mg/kg/day)	Duration (weeks)
Rat (Sprague-Dawley)	SC continuous infusion	0, 0.1, 1.0	4W 2W recovery at 0 and 1 mg/kg
Rat (Sprague-Dawley, Hsd:SD)	SC bolus	0, 0.1, 0.5, 1.0	26 W, no recovery
Rat (Crl:CD® [SD]BR/ VAF/Plus™)	IV bolus	0, 0.1, 1.0, 3.0	4W 4W recovery at 0 and 3 mg/kg
Cynomolgus monkey	SC bolus	0, 0.1, 1.0, 3.0	4W + 4W recovery
Cynomolgus monkey	IV bolus	0, 3, 10→5 (dose reduction due to injection site reactions)	4W + 4W recovery

W: weeks

Table 4-2 Reproductive toxicology studies in rhesus macaque monkeys

Study type	Route	No. of animals per group	Dose per day (mg/kg)	Treatment period
Embryofetal development	IV infusion	4-6F	0, 7.5, 75	D1-30 pc
Prenatal	IV infusion	4F	0.1, 2	GD147 single infusion
Peri-and post natal	IV infusion	5-6F	0, 0.2 and 2	GD150 until delivery
Maternal follow up after perinatal exposure	NA	5-6F	Dosed in previous pregnancy	Maternal follow up through next breeding season
Postnatal follow up after perinatal exposure in utero	Exposed in utero after IV infusion to dams	2-4F 2-4M	NA	Offspring follow up for 12 months postnatally

NA: not applicable – animals were previously exposed; GD: Gestational Day

Toxicology: Serelaxin was well tolerated in toxicology studies (Table 4-1). Although there was evidence of pharmacologic activity, no issues of toxicological significance were identified that would preclude approval of serelaxin for treatment of acute heart failure. Mild injection site reactions were observed in monkeys but were reversible. The NOAELs were the highest doses evaluated.

Safety pharmacology: In the conducted safety pharmacology studies, treatment with serelaxin did not result in any adverse effects on heart rate, blood pressure or ECG intervals at doses up to 100 times the clinical dose of 30 µg/kg/day, and did not show any biologically significant inhibition of ion-channels, including the hERG channel, *in vitro*.

Genotoxicity: Due to its protein nature, serelaxin is unlikely to enter the nucleus and interact directly with DNA. Therefore in accordance with ICH S10 guidance no studies assessing genotoxic potential of serelaxin were conducted.

Carcinogenicity: Serelaxin proposed treatment is a 48 hour infusion and thus according to guidance S1a, no carcinogenicity studies were required. Furthermore, serelaxin is identical to a naturally occurring human hormone that is present in healthy adults, and is therefore not likely to be inherently carcinogenic.

Reproductive toxicity: Rhesus monkeys were used for the evaluation of developmental and reproductive toxicity of exogenous relaxin (Table 4-2). These data are adequate to support the developmental and reproductive safety of short term serelaxin use in acute heart failure patients.

Juvenile toxicity: No specific juvenile toxicology studies were conducted due to the absence of any target organ toxicity or differences in relaxin receptor expression that suggested there might be age-related differences in sensitivity to relaxin.

Toxicokinetics and immunogenicity: Following IV administration, serelaxin distributes to blood as well as highly perfused organs, including kidneys and liver, and is cleared rapidly from serum with a tri-exponential decay over time in all animal species studied.

Serelaxin is identical to the human relaxin (H2) sequence. The relaxin sequence is highly variable across species and these sequence differences probably contribute to the fact that serelaxin is immunogenic in animals. The incidence of anti-serelaxin antibody formation was high in the toxicology studies, and these antibodies sometimes neutralized the pharmacology of serelaxin in an in vitro bioactivity assay. Nonetheless anti-serelaxin antibodies were not considered to meaningfully impact the validity of the toxicology studies, as even in the presence of these antibodies there was still evidence of sustained pharmacodynamic activity in vivo.

Local tolerance: Injection site induration was observed in monkeys when serelaxin was repeatedly administered by the IV route. These effects were observed to some degree in the vehicle animals, which received the same dose volume, but were exacerbated in the treated groups. Injection site reactions have not, however, presented an issue in clinical trials of serelaxin for AHF.

In summary, serelaxin was well tolerated in safety pharmacology studies, general toxicology studies, and developmental and reproductive toxicology studies. No safety signals were identified which would indicate a particular hazard for patients.

5 Human Pharmacokinetics

5.1 Pharmacokinetics following IV administration

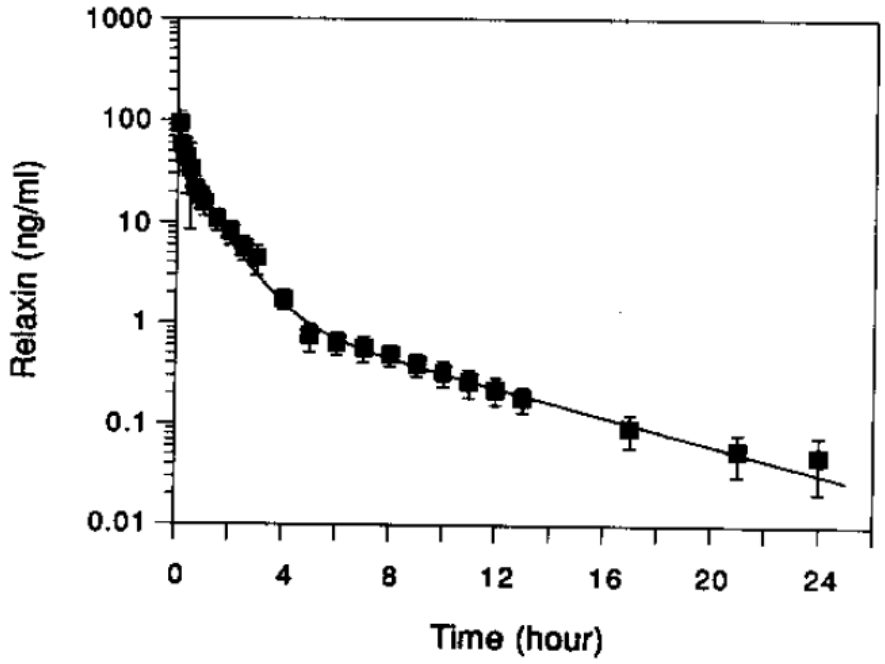
The pharmacokinetics (PK) of serelaxin have been characterized in healthy subjects and in the relevant patient populations (AHF and CHF) in 8 clinical studies across dose rates ranging from 10 to 960 µg/kg/day following the intended route of administration (IV bolus injection or IV infusion for 20-48 hours). PK data were analyzed both by a non-compartmental method and by a compartmental population PK approach.

Following a single 10 µg/kg IV bolus injection, serelaxin exhibited tri-exponential decay with the first two phases accounting for 85% of the AUC for serum concentration. Clearance (CL) was rapid with a mean residence time of 1.6 hours.

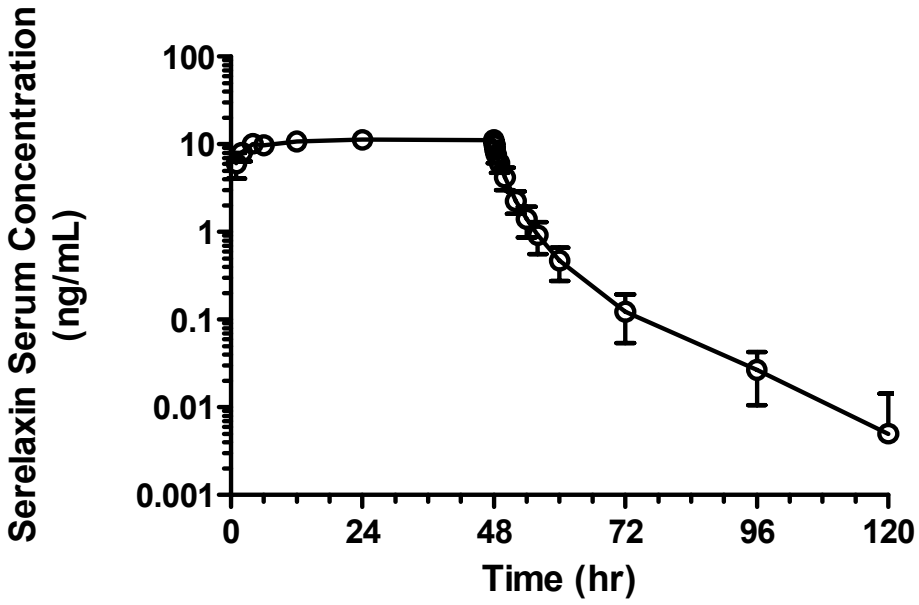
Following continuous IV infusion, serum concentration of serelaxin increased rapidly within the first few hours then gradually approached the steady state concentration (Css) starting as early as 4 to 6 hours. Upon cessation of infusion, concentration declined rapidly following a similar pattern as the IV bolus PK profile as shown in Figure 5-1.

Figure 5-1 Serelaxin serum concentration-time profiles following (a) a single IV bolus administration of 10 µg/kg in healthy subjects (study R0006g) (b) a single 48-hr IV continuous infusion of 30 µg/kg/day in healthy subjects (study CRLX030A2103)

(a) Study R0006g (mean±SD; n=25): 10 µg/kg single IV bolus, Cmax=97.6±29.1 ng/mL, tri-exponential decay: T1/2=0.09, 0.92 and 4.6 hr respectively.



(b) Study CRLX030A2103 (mean±SD; n=8; Caucasian): 30 µg/kg/day 48-hr IV infusion, Css(C48hr)=11.2±1.72 ng/mL, terminal T1/2=9.08±2.75 hr.



Across all studies, serelaxin generally exhibited linear and dose-independent PK across the range of IV doses studied. There was no evidence of time-dependent kinetics over the 20-48 hour period. A maximum mean concentration of 512±269 ng/mL was attained at the highest dose rate of 960 µg/kg/day. Steady state geometric mean concentration of 17.7 ng/mL at a nominal dose of 30 µg/kg/day was attained in the pivotal Phase III study RELAX-AHF.

Serelaxin has shown comparable steady state exposure and systemic clearance following IV administration to healthy volunteers and heart failure patients. The apparent volume of distribution at steady state (Vss) was higher in AHF patients compared to that in healthy subjects, possibly due to fluid overload in the patient population.

5.2 Pharmacokinetics in special populations

The PK of serelaxin were unaffected by hepatic impairment (mild, moderate, and severe hepatic impairment based on Child-Pugh scores ranging from 5 to 15) when compared with matched healthy subjects with normal hepatic function following continuous 24-hour IV infusion at 30 µg/kg/day. Impairment of renal function (mild and moderate, determined by estimated GFR [eGFR] 30-89 mL/min/1.73m^2) did not significantly affect serelaxin systemic CL in patients with AHF. Existing data are limited in subjects with severe renal impairment.

Gender, age (>65 yr), race or ethnicity, and body weight did not appear to have a significant effect on the CL of serelaxin. An ethnic sensitivity study in healthy Japanese and Caucasian subjects did not reveal any clinically meaningful PK differences between the two ethnic groups.

5.3 Drug-drug interactions

No formal PK and pharmacodynamics (PD) drug-drug interaction (DDI) studies were conducted given the low risk of PK DDI based on a mechanism-driven risk-based analysis and the low risk of PD interactions based on the the short infusion time of 48 hours. Concomitant medications most commonly used in the standard of care in patients with AHF in the hospital or emergency room setting did not alter the Css or the CL of serelaxin. There was no clinical indication of AEs related to DDI. As a therapeutic protein, serelaxin is expected to be cleared in the body via catabolism by proteases/peptidases which have no direct interaction with cytochrome P450 enzymes. To support the mechanism-driven assessment for PK DDIs, cytokines were measured in RELAX-AHF. No statistically significant changes were observed in levels of pro-inflammatory cytokines, including interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), supporting the concept that no cytokine-mediated therapeutic protein-drug interactions are expected.

Although there is a theoretical risk of DDI due to the increased creatinine clearance following serelaxin treatment (CRLX030A2201), the observed increase was transient in duration, which is unlikely to have any significant impact on the steady state exposure of the concomitant medications that are intented for chronic use. Therefore the theoretical risk of potential DDI due to increased creatine clearance is considered low and unlikely to result in the need for any clinically meaningful dose adjustment for concomitant medications.

With regards to PD DDIs, the efficacy observed in RELAX-AHF was not affected by standard of care medications including ACEi, ARBs, BBs, MRA and IV nitrates. Conversely the potential of serelaxin to affect the PD of other commonly used drugs is low based on the short infusion period.

6 Hemodynamic Studies

Following the initial pilot single-center, open label safety and PD study RLX.CHF.001, hemodynamic effects of serelaxin were evaluated in the multi-center, double-blind, placebo controlled study CRLX030A2201. Renal hemodynamic effects were assessed in study CRLX030A2202, creatinine clearance in CRLX030A2201, and renal function parameters including serum creatinine, uric acid and BUN in all HF studies. The dose rate of 30 µg/kg/day for studies CRLX030A2201 and CRLX030A2202 was selected to match that of the RELAX-AHF study, which was based on the results of the Pre-RELAX-AHF study (see Section 7). For feasibility reasons, the duration of infusion had to be limited to 20 and 24 hours, respectively, in these two hemodynamic studies.

6.1 Systemic hemodynamic studies in heart failure patients

6.1.1 Pilot study RLX.CHF.001

The pilot, open-label study RLX.CHF.001 enrolled 16 patients with compensated CHF. The study had no concurrent control and applied an intra-subject dose escalation spanning serelaxin 10-960 µg/kg/day over 8 hours for each dose rate. A maximum reduction in PCWP from baseline of 5.0 ±2.6 mmHg (mean ±SD) at 8 hours of serelaxin 30 µg/kg/day was observed. Effects on mean PAP were similar, with a decrease of 4.5 ±4.2 mmHg (mean ±SD) at 8 hours of serelaxin 30 µg/kg/day. There was a trend toward increasing CI with increasing dose/duration of treatment resulting in a mean change from baseline of 0.25 ±0.4 (mean ±SD) L/min/m^2 at 8 hours of serelaxin 30 µg/kg/day. The change from baseline in SVR was -171 ±159 dynes*s/cm^5 (mean ±SD) at 8 hours of serelaxin 30 µg/kg/day. At dose rates of serelaxin ranging from 10 to 960 µg/kg/day in CHF patients, early decreases in serum creatinine (within 8 hours), uric acid and BUN were observed during the 24-hour serelaxin IV infusion and for an additional 24 hours thereafter. At the highest dose rate of serelaxin (960 µg/kg/day), one week after completion of dosing, small, clinically insignificant increases in creatinine (mean change 0.263 ± 0.109 mg/dL) and BUN (mean change 7 ±13.78 mg/dL) were observed.

6.1.2 Study CRLX030A2201

In study CRLX030A2201, a randomized, double-blind, parallel-group, placebo-controlled Phase II study, 71 patients hospitalized for AHF with a mean pulmonary capillary wedge pressure (PCWP) ≥18 mmHg, SBP ≥115 mmHg, eGFR ≥30 mL/min/1.73m^2 and treated with IV loop diuretics (40 to 120 mg furosemide or equivalent) prior to randomization, received a 20-hour IV infusion of serelaxin at 30 µg/kg/day or placebo within 48 hours from admission/presentation. Hemodynamic parameters were assessed during and for 4 hours after the end of the IV infusion. The primary objective was to evaluate the hemodynamic responses in PCWP and cardiac index (CI) to 30 µg/kg/day IV serelaxin or placebo in the first 8 hours in patients hospitalized with AHF. Secondary objectives included the effects of serelaxin on the onset and offset of the hemodynamic effects, PK, renal function parameters, safety and tolerability.

Baseline mean ±SD PCWP was similar between treatment groups at 26.1 ±5.86 mmHg in the serelaxin group and 26.5 ±5.17 mmHg in the placebo group. Serelaxin infusion was associated with a decrease in peak PCWP from baseline over the first 8 hours of 6.69 ±0.59 mmHg vs. 4.25 ± 0.60 mmHg in the placebo group (both LSmean ±SE) and the treatment difference was -2.44 mmHg (95% CI: -4.10 to -0.78 mmHg; p=0.0040, see Table 6-1). This result is supported by the significant treatment difference in the time-weighted average PCWP change from baseline over 0-8 hours of -2.70 mmHg (p=0.0001). The treatment differences in the time weighted average PCWP change from baseline over 8-20 hours (p=0.0322) and 0-20 hours (p=0.0042) were also statistically significant in favor of serelaxin.

Table 6-1 Statistical analysis of PCWP (mmHg) - PD analysis set

Parameter	Placebo LSmean (SE) (mmHg) (N=31)	Serelaxin 30 µg/kg/day LSmean (SE) (mmHg) (N=32)	Difference (mmHg) [95% CI]	p-value
Change from baseline in peak PCWP over 8 h	-4.25 (0.60)	-6.69 (0.59)	-2.44 [-4.10, -0.78]	0.0040
Time-weighted average change from baseline				
0-8 h based on AUEC0-8h	-1.08 (0.51)	-3.79 (0.50)	-2.70 [-4.10, -1.31]	0.0001
8-20 h based on AUEC8-20h	-2.67 (0.74)	-4.90 (0.73)	-2.24 [-4.28, -0.19]	0.0322
0-20 h based on AUEC0-20h	-2.04 (0.60)	-4.46 (0.59)	-2.42 [-4.08, -0.76]	0.0042
20-24 h based on AUEC20-24h	-3.11 (0.85)	-4.41 (0.83)	-1.30 [-3.63, 1.03]	0.2733

Results are from an ANCOVA model with treatment as the classification factor and baseline as a covariate

PCWP: pulmonary capillary wedge pressure; AUEC: area under the effect curve; SE: standard error of the mean

The onset of serelaxin's effect was evident at 30 minutes with a significant treatment difference at 2 hours after the start of the infusion and statistically significant differences in PCWP change from baseline between the treatment groups were observed at 2, 4, 6 and 8 hours from the start of infusion. In the serelaxin group, the largest treatment difference in PCWP change from baseline was -3.93 mmHg at 4 hours after the start of infusion (95% CI: -6.00 to -1.86 mmHg; p=0.0002). The effect on PCWP was no longer statistically significant during the 4 hour wash-out.

Serelaxin also resulted in a mean PAP decrease from baseline during the first 8 hours of 7.56 ±0.72 mmHg vs. 3.63 ±0.74 mmHg in the placebo group (both LSmean ±SE) and resulted in a treatment difference of -3.93 mmHg (95% CI: -5.96 to -1.90 mmHg; p=0.0001, see Table 6-2). Similar results were observed for the time weighted average mean PAP change from baseline over 0-8 hours, with a statistically significant treatment difference (p<0.0001). There was also a statistically significant treatment difference for the time weighted average change from baseline over 8-20 hours (p=0.0028) and 0-20 hours (p=0.0002). The onset was rapid with a significant treatment difference in mean PAP change from baseline at 30 minutes after the start of infusion. At 0.5, 2, 4, 6 and 8 hours and after the end of infusion at 21 hours, the treatment differences in mean PAP change from baseline were statistically significant. The largest treatment difference in mean PAP change from baseline of -5.17 mmHg occurred 4 hours after the start of infusion (95% CI: -7.49 to -2.86 mmHg; p<0.0001).

Table 6-2 Statistical analysis of mean PAP (mmHg) – PD analysis set

Parameter	Placebo LSmean (SE) (mmHg) (N=31)	Serelaxin 30 ug/kg/day LSmean (SE) (mmHg) (N=32)	Difference [95% CI]	p-value
Change from baseline in peak mean PAP over 8 h	-3.63 (0.74)	-7.56 (0.72)	-3.93 [-5.96, -1.90]	0.0001
Time weighted average change from baseline in mean PAP				
0-8 h based on AUEC0-8h	0.06 (0.66)	-3.98 (0.65)	-4.04 [-5.86, -2.22]	<0.0001
8-20 h based on AUEC8-20h	-0.80 (0.89)	-4.56 (0.88)	-3.76 [-6.22, -1.29]	0.0028
0-20 h based on AUEC0-20h	-0.45 (0.73)	-4.32 (0.72)	-3.87 [-5.89, -1.86]	0.0002
20-24 h based on AUEC20-24h	-1.67 (0.98)	-4.29 (0.96)	-2.62 [-5.31, 0.07]	0.0561

Results are from an ANCOVA model with treatment as the classification factor and baseline as a covariate

PAP: Pulmonary arterial pressure; AUEC: area under the effect curve; LSmean: least squares mean; SE: standard error.

Baseline mean (±SD) CI was 2.37 (±0.70) L/min/m^2in the serelaxin group and 2.17 (±0.62) L/min/m^2 in the placebo group. There was no significant treatment difference in changes from baseline during the first 8 hours in study CRLX030A2201

SVR was also reduced by serelaxin with the treatment difference of -136.91 dynes*sec/cm^5 (95% CI: -261.89 to -11.93 dynes*sec/cm^5) in the time-weighted average SVR change from baseline over 0-8 hours (p=0.0318 vs. placebo). The peak SVR change from baseline was -368.1 ±45.9 dynes*sec/cm^5 (LSmean ±SE) in the serelaxin group over the first 8 hours. A statistically significant treatment difference was also observed for the time-weighted average SVR change from baseline over 20-24 hours (p=0.0087).

A summary of peak change and time weighted average change from baseline over the first 8 hours for CI, CO, SVR, RAP, systolic and diastolic PAP is provided in Table 6-3 and Table 6-4.

Table 6-3 Statistical analysis of peak change from baseline over 8 hours for other hemodynamic parameters - PD analysis set

Parameter	Peak change from baseline over 8 hours			
	Placebo LSmean (SE) (N=31)	Serelaxin 30 µg/kg/day LSmean (SE) (N=32)	Difference [95% CI]	p-value
Cardiac index (L/min/m^2)	0.30 (0.05)	0.32 (0.05)	0.02 [-0.13, 0.16]	0.7936
Cardiac output (L/min)	0.59 (0.11)	0.62 (0.11)	0.04 [-0.26, 0.33]	0.8105
SVR (dynes*sec/cm^5)	-284.62 (45.92)	-368.06 (45.92)	-83.44 [-211.72, 44.85]	0.2024
RAP (mmHg)	-2.07 (0.36)	-3.24 (0.36)	-1.16 [2.16, -0.17]	0.0216
Systolic PAP (mmHg)	-4.59 (1.05)	-10.77 (1.03)	-6.19 [-9.07, -3.30]	<0.0001
Diastolic PAP (mmHg)	-3.89 (0.71)	-6.50 (0.70)	-2.62 [-4.58, -0.66]	0.0089

Results are from an ANCOVA model with treatment as the classification factor and baseline as a covariate

SVR: systemic vascular resistance; RAP: right arial pressure; PAP: pulmonary arterial pressure; CI: confidence interval; LSmean: least squares mean; SE: standard error.

Table 6-4 Statistical analysis of time-weighted average change from baseline over 0- 8 hours for other hemodynamic parameters - PD analysis set (based on AUEC 0-8 hours)

Parameter	Placebo LSmean (SE) (N=31)	Serelaxin 30 µg/kg/day LSmean (SE) (N=32)	Difference [95% CI]	p-value
Cardiac index (L/min/m^2)	0.07 (0.04)	0.12 (0.04	0.04 (-0.07, 0.15)	0.4754
Cardiac output (L/min)	0.15 (0.08)	0.23 (0.08)	0.07 (-0.15, 0.30)	0.5178
SVR (dynes*sec/cm^5)	-29.24 (44.73)	-166.15 (44.73)	-136.91 (-261.80, -11.93)	0.0318
RAP (mmHg)	-0.23 (0.36)	-1.12 (0.36)	-0.89 (-1.89, 0.12)	0.0838
Systolic PAP (mmHg)	0.64 (0.94)	-5.35 (0.93)	-5.99 (-8.59, -3.39)	<0.0001
Diastolic PAP (mmHg)	0.22 (0.60)	-3.29 (0.59)	-3.08 (-4.73, -1.42)	0.0003

Results are from an ANCOVA model with treatment as the classification factor and baseline as a covariate

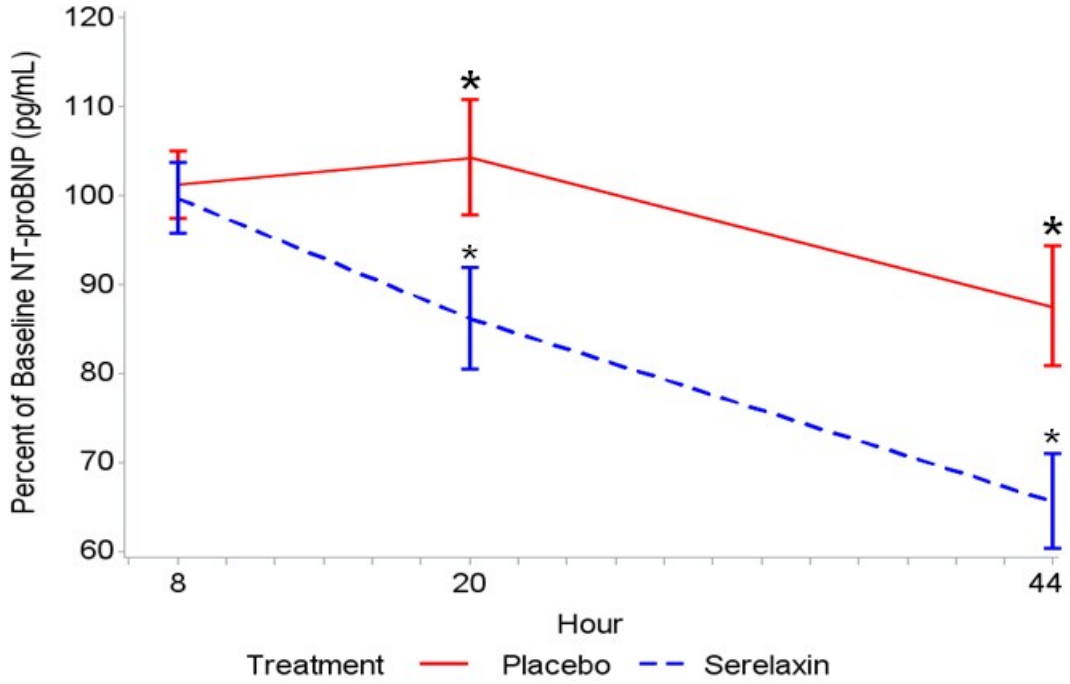
SVR: systemic vascular resistance; RAP: right atrial pressure; PAP: pulmonary arterial pressure; AUEC: area under the effect curve; CI: confidence interval; LSmean: least squares mean; SE: standard error.

As expected, changes in SBP and DBP were larger in the serelaxin than the placebo group. The mean treatment differences were -6.25 mm Hg for SBP and -8.86 mmHg for DBP at the end of the infusion (20 hours). Based on the study protocol specificied criteria for SBP changes requiring dose adjustment or discontinuation, three patients in the serelaxin group had their infusion stopped and the infusion rate was reduced in one patient in the placebo group.

Creatinine clearance increased over the infusion period with serelaxin. A post-hoc ANCOVA confirmed a statistically significant treatment difference of 39% (ratio of geomean ratios: 1.39; 95% CI: 1.07 to 1.81; p=0.0143 vs. placebo) for 0-20 hours in creatinine clearance change from baseline.

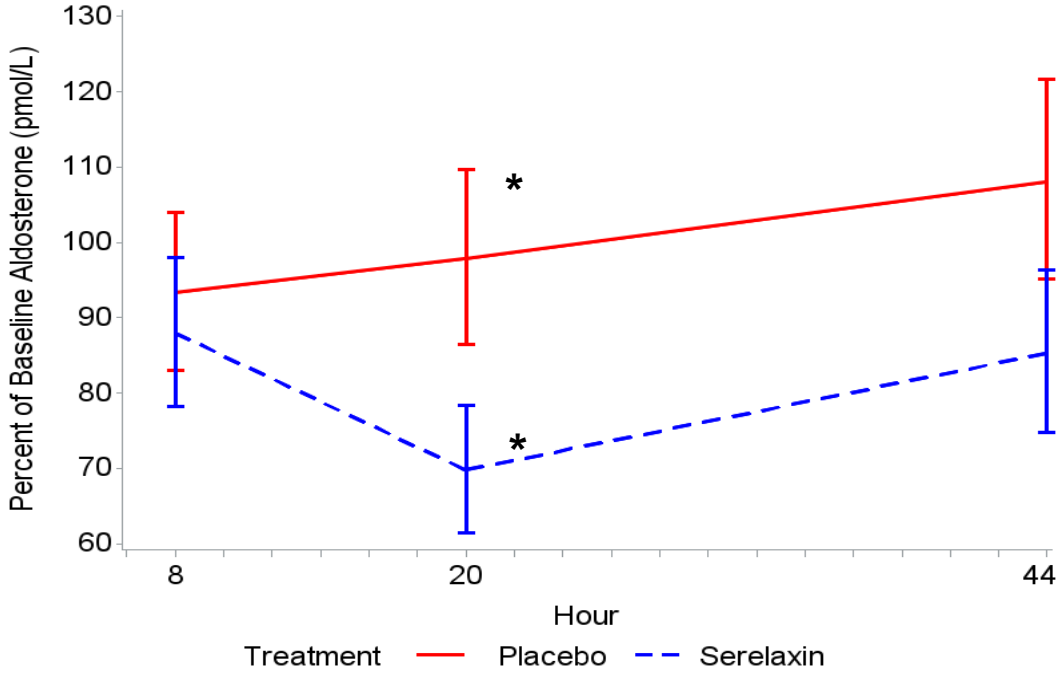
The positive changes in hemodynamic parameters are accompanied by changes in biomarkers, including greater decreases from baseline in NT-proBNP and plasma aldosterone in the serelaxin group. Results of NT-proBNP and plasma aldosterone as percent of baseline values are presented in Figure 6-1 and Figure 6-2, respectively. Serelaxin infusion was well-tolerated in this study.

Figure 6-1 Percent of baseline in geometric least square mean (±SE) plasma NT-proBNP - PD analysis set (N=63)



*p<0.05 (post hoc ANCOVA comparing the two trreatments for log transformed ratio to baseline)

Figure 6-2 **Percent of baseline in geometric least square mean (±SE) plasma aldosterone - PD analysis set (N=63)**



*p<0.05 (post hoc ANCOVA comparing the two treaments for log transformed ratio to baseline)

6.2 Renal hemodynamics in chronic heart failure patients

In study CRLX030A2202, a randomized, double-blind, parallel-group, placebo-controlled Phase II study, 87 CHF patients with BNP ≥100 pg/mL or NT-proBNP ≥400 pg/mL, NYHA class II-III, reduced left ventricular ejection fraction (≤45%), worsening symptoms in the last 3 months, SBP ≥110 mmHg, eGFR 30 to 89 mL/min/1.73m^2 and treated with a stable loop diuretic dose (40 to 240 mg furosemide p.o.) received a 24-hour IV infusion of serelaxin treatment at 30 µg/kg/day or placebo. The primary objective was to evaluate the effects of IV 30 µg/kg/day serelaxin or placebo for 24 hours on renal plasma flow (RPF) and GFR over the 8-24 hour interval. Secondary objectives included the effects of serelaxin on filtration fraction, renal function parameters, safety and tolerability.

The results of the study are presented in Table 6-5. A statistically significant treatment difference of 13% in the RPF change from baseline over 8-24 hours was observed with serelaxin (p=0.0386). The change from baseline over 8-24 hours was 29% in the serelaxin group and 14% in the placebo group. Differences in RPF in the treatment groups were also statistically significantly in favor of serelaxin for change from baseline in RPF over 0-24 and 24-28 hours, with treatment differences of 16% (p=0.0042) and 16% (p=0.0115), respectively. The onset was rapid, present at 2 hours, with a maximum RPF increase from baseline of 56% occurring at 4 hours after the start of the serelaxin infusion vs. 20% in the placebo group. At individual time points, the treatment differences for change from baseline in RPF were statistically significant in favor of serelaxin at hour 2, 4, 6, 8, 24, and 26, with treatment differences ranging from 14% to 30%.

In this study, changes in RPF were not accompanied by changes in GFR. The lack of effect on GFR results may be a consequence of the study population (compensated CHF patients). Treatment differences in filtration fraction over 8-24 hours, 0-24 hours and 24-28 hours were statistically significant when comparing serelaxin to placebo group (ranging between -16 to -22%), with smaller increases from baseline for serelaxin. Overall, safety results were consistent with results from other clinical studies.

Table 6-5 Statistical analysis of time weighted average change for renal hemodynamic - PD analysis set

Parameter	Placebo LS Geomean Ratio to Baseline (SE) (N=37)	Serelaxin 30 µg/kg/day LS Geomean Ratio to Baseline (SE) (N=28)	Ratio of LS Geomean Ratios# [95% CI]	p-value
RPF				
0-24 hours	1.13 (1.04)	1.31 (1.05)	1.16 (1.05, 1.28)	0.0042
8-24 hours	1.14 (1.05)	1.29 (1.05)	1.13 (1.01, 1.27)	0.0386
24-28 hours	1.16 (1.05)	1.35 (1.05)	1.16 (1.03, 1.30)	0.0115
GFR				
0-24 hours	1.66 (1.04)	1.60 (1.04)	0.96 (0.88, 1.06)	0.4336
8-24 hours	1.87 (1.05)	1.78 (1.06)	0.95 (0.84, 1.07)	0.3932
24-28 hours	2.13 (1.05)	1.92 (1.06)	0.90 (0.79, 1.03)	0.1371
FF				
0-24 hours	1.44 (1.04)	1.20 (1.05)	0.84 (0.76, 0.92)	0.0004
8-24 hours	1.62 (1.05)	1.36 (1.05)	0.84 (0.75, 0.94)	0.0019
24-28 hours	1.81 (1.05)	1.41 (1.06)	0.78 (0.69, 0.88)	<0.0001

Results are from an ANCOVA model with terms for treatment, diuretic dose stratum and baseline. Values are log-transformed prior to analysis. Baseline is the value at Time 0.

serelaxin to placebo; RPF: renal plasma flow; GFR: glomerular filtration rate; FF: filtration fraction; CI: confidence interval; LS-Geomean: geometric least-squares mean; SE: standard error

6.3 Implications of hemodynamic study results

Results of CRLX030A2201 support serelaxin's proposed mechanism of action and effects on pre- and afterload leading to improved cardiac workload. These hemodynamic effects of serelaxin shown in the study are consistent with the observed symptomatic improvement in RELAX-AHF study (Section 7). Results of study CRLX030A2202 support serelaxin's effect on renal perfusion.

7 RELAX-AHF Phase II/III Program

7.1 Overview

The RELAX-AHF clinical program included a Phase II dose-ranging study Pre-RELAX-AHF and a Phase III pivotal study RELAX-AHF that utilized the same study design and enrollment criteria to evaluate the efficacy and safety of 48-hour infusion of serelaxin for the treatment of hospitalized AHF patients.

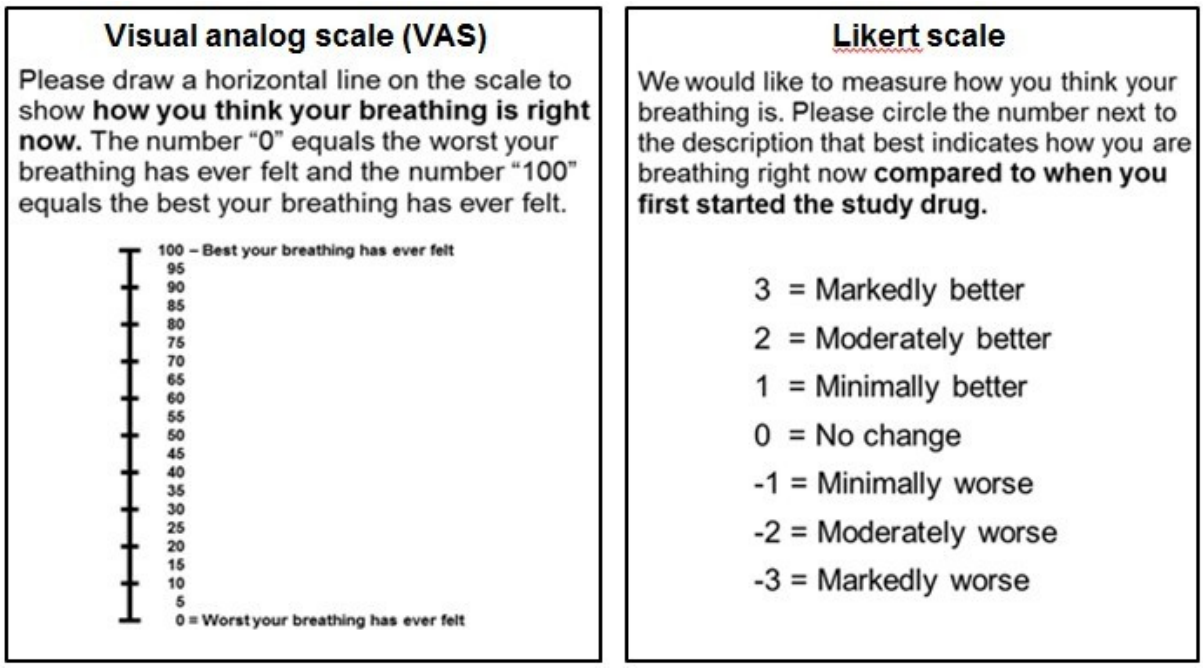
- The Phase II/III studies enrolled AHF patients who presented to the hospital with sustained dyspnea following treatment with IV furosemide ≥40 mg, and all patients had normal to elevated blood pressure, increased BNP and/or NT-proBNP, and mild to moderate renal dysfunction. The patients enrolled in Pre-RELAX-AHF and RELAX-AHF studies represent a large proportion of AHF patients (Adams et al 2005, Gheorghiade et al 2006a, Gheorghiade et al 2006b, Gheorghiade and Pang 2009). In addition, patients were randomized within 16 hours from initial presentation.
- The program included approximately 1,400 AHF patients randomized from 11 countries (including the US), with patient characteristics similar to those of US AHF patient registries.
- The guideline-recommended AHF treatments were allowed as background therapy for the studies, e.g., use of IV loop diuretics was permitted at anytime from patient presentation during the study, and IV nitrates could be used (at a dose of ≤0.1 mg/kg/hr) prior to randomization if the patient had a systolic BP >150 mmHg at screening. There was no restriction for HF treatment post-randomization, and they could be administered if clinically warranted per local and/or institutional standards. Such treatments included vasodilators, inotropic agents, vasopressors or mechanical/circulatory support.
- The studies were designed to evaluate the treatment effects of serelaxin on both the short-term and long-term clinically important endpoints for AHF. These endpoints included patient-reported outcome (PRO)-based assessment of dyspnea relief, in-hospital WHF and post-discharge clinical outcomes.

7.1.1 Assessing dyspnea endpoint with Patient-Reported Outcome scales

PRO-based assessment of dyspnea relief was pre-defined as the key efficacy endpoint in the serelaxin clinical program. Change in dyspnea was assessed using two PRO instruments: the 100-mm Visual Analog Scale (VAS) and 7-point Likert Scale (Figure 7-1):

- The first primary endpoint was based on the area under curve (AUC) of absolute change from baseline in VAS scores. This endpoint assessed both the improvement and worsening in dyspnea through Day 5. The endpoint was designed to capture the occurrence of in-hospital WHF as an integral component.
- The second primary endpoint was based on the categorical 7-point Likert scale, and it assessed the occurrence of moderate/marked improvement in dyspnea through Day 1 (requiring improvement at all three visits at 6, 12 and 24 h); it did not capture worsening of symptoms or the occurrence of in-hospital WHF.

Figure 7-1 Patient-reported outcome (PRO) scales for dyspnea assessment



7.1.2 Reporting of WHF events in RELAX-AHF and relevance for the primary dyspnea endpoint

7.1.2.1 Definition of WHF event in the RELAX-AHF program

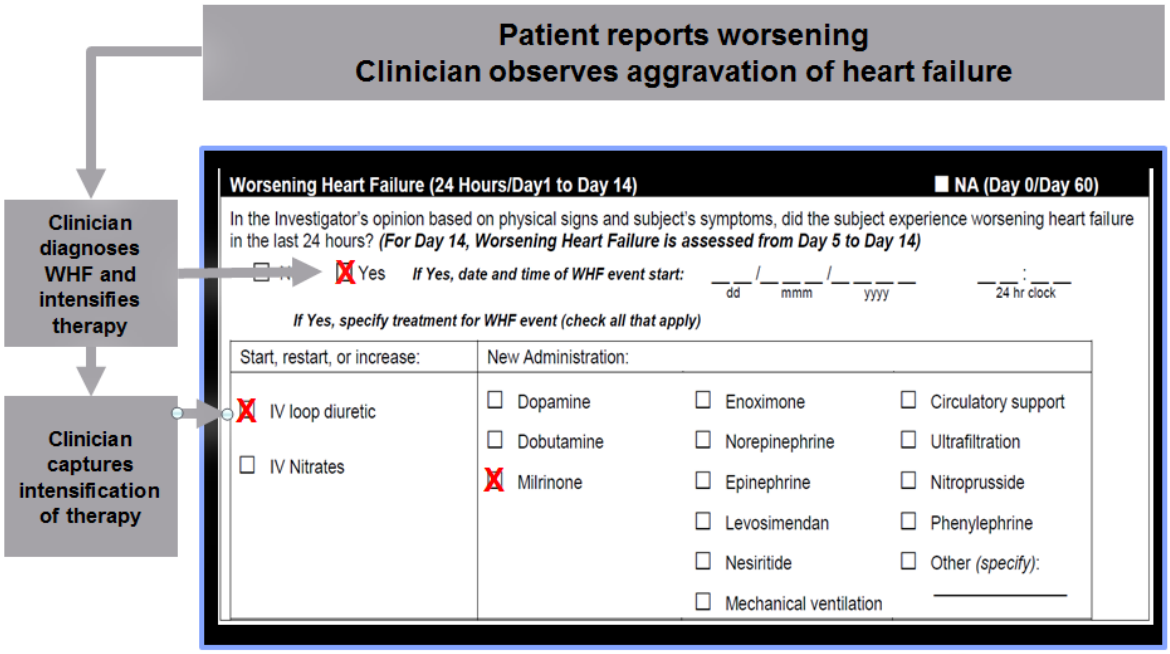
In-hospital WHF events are episodes of acute symptomatic decompensation and represent a severe manifestation of the AHF disease continuum requiring immediate intensification of rescue therapy. A WHF event fundamentally changes the patient's in-hospital clinical course and is associated with worse long-term clinical outcomes. The definition of WHF events, according to the RELAX-AHF study protocol, required the occurrence of worsening signs and symptoms of HF, which was treated by the immediate administration of rescue therapy:

"WHF is defined for this study as worsening signs and/or symptoms of heart failure that require an intensification of intravenous therapy for heart failure or mechanical ventilatory or circulatory support. Such treatment can include the institution or up-titration of IV furosemide, IV nitrates or any other IV medication for heart failure, or institution of mechanical support such as mechanical ventilation, intra-aortic balloon pump (IABP), etc. (Cotter et al 2010; Weatherley et al 2009)."

The above definition of a WHF event in RELAX-AHF is consistent with the definition of WHF used in recent randomized AHF trials VERITAS (McMurray et al 2007), PROTECT (Massie et al 2010, Metra et al 2011), REVIVE-2 (Packer et al 2013), and TRUE-AHF (clinicaltrials.gov).

In the RELAX-AHF program, the determination of WHF was made by the physician on clinical grounds based on patient report and on investigators' assessment of HF signs and symptoms (Figure 7-2). It is important to note that the identification of WHF was not primarily related to the administration of additional IV therapy. Instead, the decision to give additional IV therapy or other interventions (e.g., mechanical/circulatory support) to manage WHF events was used as a confirmation that the investigator considered the patient's deterioration to be sufficiently severe to warrant rescue intervention(s).

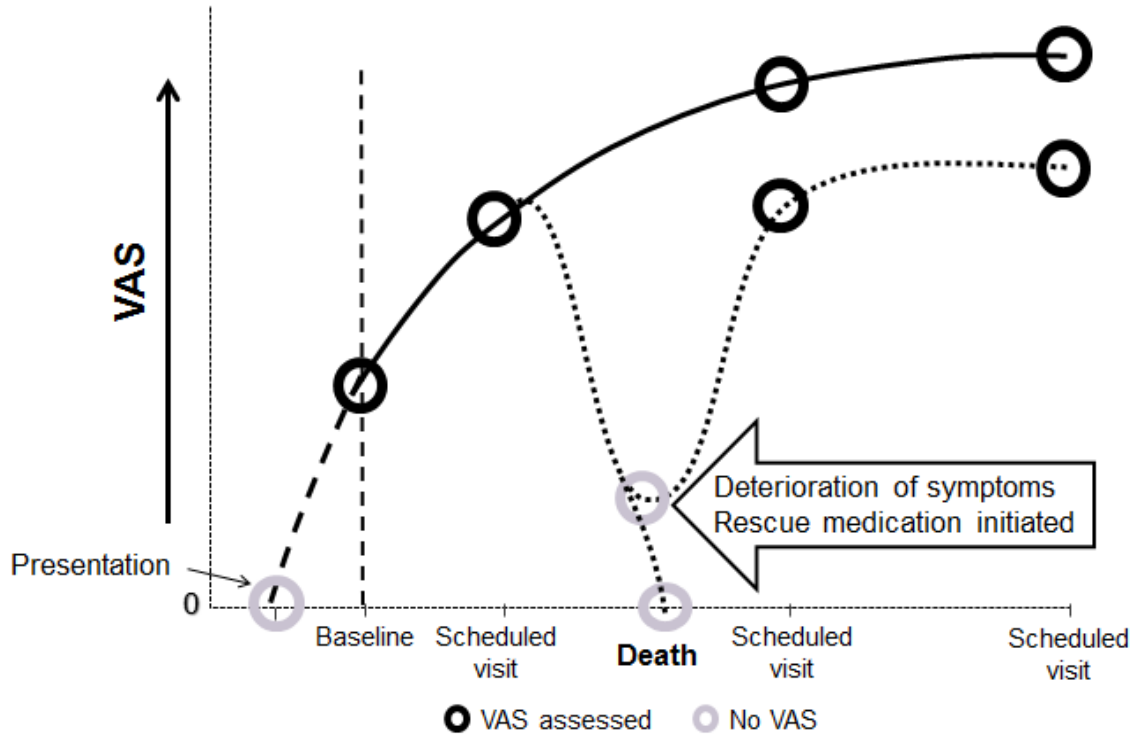
Figure 7-2 Collection of WHF events in case report form



7.1.2.2 Dyspnea score assignment to incorporate WHF events as an integral component of the primary dyspnea endpoint

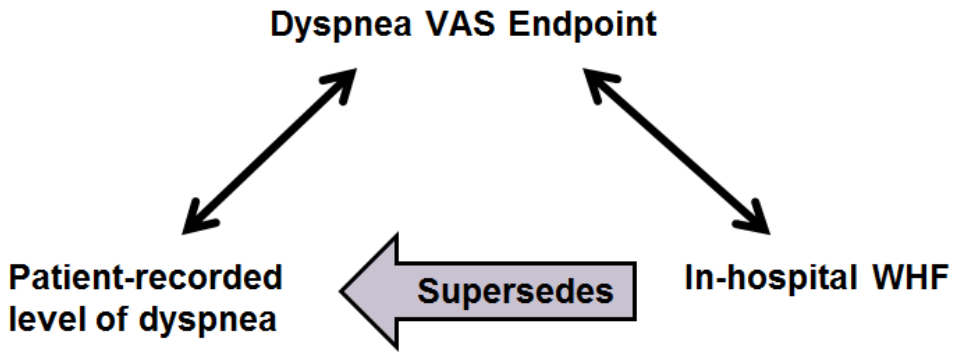
A representative hypothetical patient clinical course for improvement (or worsening) of dyspnea based on VAS assessments and an intercurrent WHF event is illustrated in Figure 7-3. It should be first recognized that patients had already received IV HF therapy by the time dyspnea was initially assessed at baseline on VAS. Thus the baseline VAS scores do not reflect the initial levels of dyspnea encountered by patients at presentation because their symptoms had improved prior to the first VAS assessment. Secondly, the patient reported dyspnea scores were captured in the RELAX-AHF program at pre-defined scheduled time points, e.g., at 6, 12, 24 hours, and then daily through Day 5 and Day 14, after the start of study drug infusion, whereas an intercurrent WHF event could occur at any time in between the scheduled dyspnea assessments. While most patients typically improve over time (shown as black line in Figure 7-3), some patients experience worsening of their signs and symptoms between scheduled assessments which are not captured by routine study assessments (see dotted line in Figure 7-3).

Figure 7-3 Schematic of a patient's clinical course with dyspnea and a WHF event



WHF (or death) was incorporated as an integral component of the VAS AUC dyspnea endpoint in the RELAX-AHF program. It was pre-specified in the study protocol that the worst-reported dyspnea score for any patient in the study would be carried forward for all time points after the onset of the WHF event, regardless of whether the score was missing or not. This analysis of the assigned dyspnea scores in the case of a WHF event (or death) was designed so that the worst score superseded the reported VAS scores after the onset of WHF (Figure 7-4).

Figure 7-4 Dyspnea VAS endpoint as pre-specfied in the RELAX-AHF protocol



Note: For patients who have a worsening heart failure event (either experience an in-hospital WHF event or die during the index hospitalization or re-hospitalization due to HF) by Day 5, the worst VAS score reported in any patient at any time point will be carried forward for all time points after the onset of event, regardless of whether the score is missing or not.

The rationale for pre-specifying in the study protocol that the worst-reported dyspnea score would be assigned in the analysis from time of onset of in-hospital WHF event was as follows:

1. By definition, the WHF event must be accompanied by worsening signs and symptoms of HF requiring urgent intervention, and it represents a severe manifestation of AHF. The WHF event reflects the occurrence of treatment failure.
2. Secondly, the intensification of rescue therapy in response to the occurrence of a WHF event distorts future assessments of the dyspnea symptoms under the originally randomized treatment. Reliance only on the reported data collected at pre-specified timepoints necessarily ignores the occurrence of serious clinical events of WHF occurring between the scheduled assessments. The value of data obtained after administration of rescue medication is discussed in various publications (O'Neill and Temple 2012; Keene 2011; Mallinckrodt et al 2012), and it is generally agreed that data obtained after receiving the rescue medication may be of limited value, especially for studies of symptom improvement.
3. Finally, the in-hospital WHF has been consistently associated with longer length of hospital stay and significantly increased risk of intermediate-term (30 days) and long-term (180 days) mortality in AHF studies (McMurray et al 2007; Metra et al 2011; Weatherley et al 2009). The association of a WHF event, regardless of type of IV rescue therapies received, with a protracted in-hospital clinical course (e.g., significantly prolonged overall hospital stay and ICU/CCU stay), and increased mortality risk was confirmed in the RELAX-AHF program.

The pre-specified dyspnea score assignment using the worst reported dyspnea score for all assessments after WHF event or death occurred in RELAX-AHF program is consistent with the designs of many other AHF trials, which also used the worst reported score and/or rank for patients who experienced a WHF event or died (Table 7-1).

Table 7-1 Score/ranking assignment for WHF in primary efficacy analysis of recently completed or ongoing AHF studies

Study	Drug	Assignment after WHF
EVEREST	Tolvaptan	No
ASCEND	Nesiritide	No
VERITAS	Tezosentan	Baseline carried forward
PROTECT	Rolofylline	Worst ranking *
REVIVE	Levosimendan	Worst ranking *
RELAX-AHF	Serelaxin	Worst score
TRUE-AHF	Ularitide	Worst ranking *

* WHF was part of a clinical composite endpoint

In summary, incorporating in-hospital WHF events or death into the primary efficacy analysis of PRO-based assessments of changes in dyspnea was prospectively designed in the study protocols of the RELAX-AHF Phase II/III program. Use of the worst-reported dyspnea score to supersede reported values after the WHF event is consistent with the fact that the in-hospital WHF event represents a patient's deteriorating clinical condition and treatment failure and requires immediate initiation of rescue therapy. A WHF event alters the in-hospital clinical course as evidenced by prolongation of the hospital stay, and it is also associated with a significantly increased risk of post-discharge mortality.

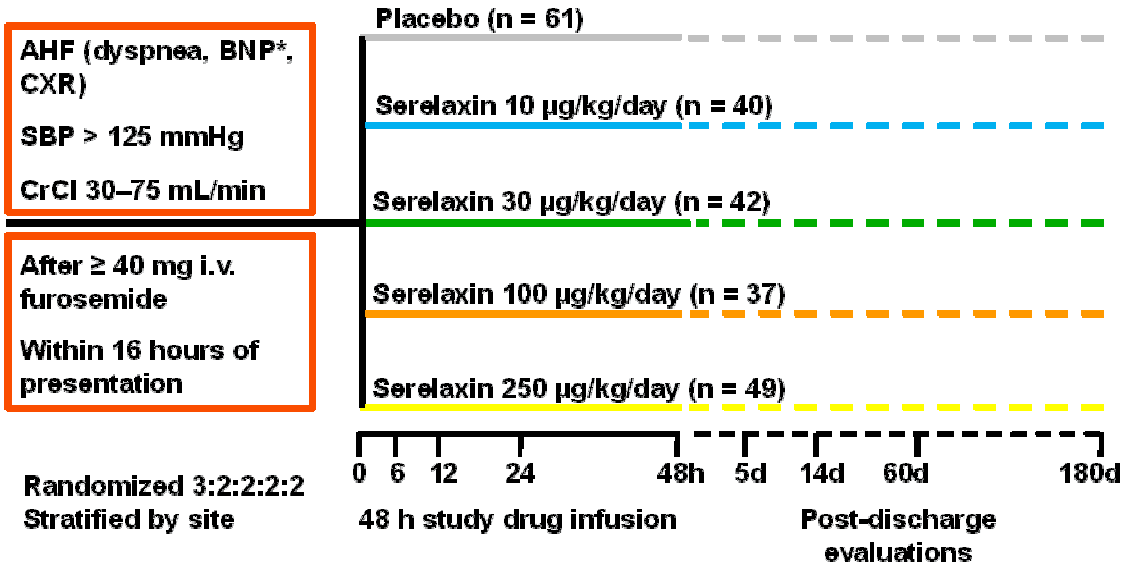
7.2 Dose-ranging Phase II study: Pre-RELAX-AHF

The relationship between various doses of IV infusion of serelaxin and their efficacy (hemodynamic and clinical endpoints) was evaluated in two studies of HF patients. Study RLX.CHF.001, a pilot safety, dose-escalation hemodynamic study, evaluated a wide dose range of serelaxin from 10 to 960 µg/kg/day in stable CHF patients. Results from this study were used to select doses for the subsequent Phase II study, Pre-RELAX-AHF (48-hour infusion at 10, 30, 100 and 250 µg/kg/day in hospitalized AHF patients).

Study Pre-RELAX-AHF was a Phase II, multicenter, randomized, double-blind, placebo-controlled study in patients hospitalized with AHF, e.g., dyspnea at rest or minimal exertion despite treatment with IV furosemide ≥40 mg, SBP >125 mmHg, elevated BNP or NT-proBNP, mild-to-moderate renal impairment, and the randomization had to occur within 16 hours from presentation (Figure 7-5). The study evaluated the effects of different doses of serelaxin on VAS and Likert dyspnea scales, in-hospital WHF, prevention of renal impairment and a range of clinical safety and efficacy endpoints. The study was designed to examine dose response relationships; it was not powered for the detection of statistical differences between individual doses for any individual endpoint.

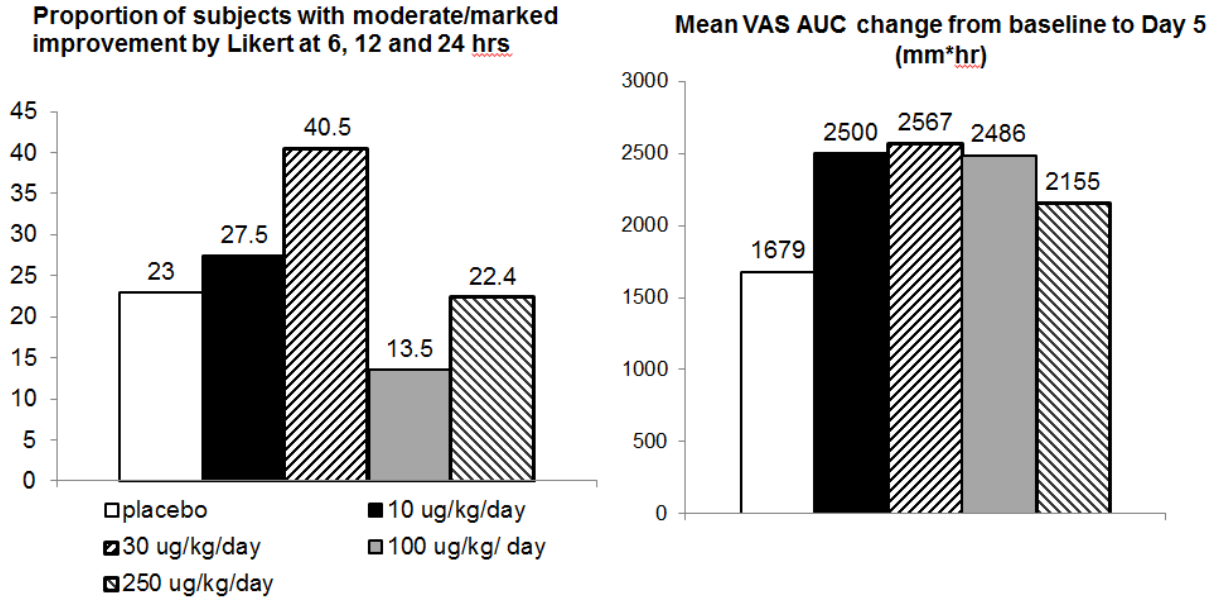
A total of 234 patients were randomized, including 229 patients (97.9%) who received continuous IV infusion for up to 48 hours, of placebo or serelaxin (10 µg/kg/day, 30 µg/kg/day, 100 µg/kg/day and 250 µg/kg/day) in a randomized ratio of 3:2:2:2:2. Patients in all treatment groups also received background therapy for AHF.

Figure 7-5 Pre-RELAX-AHF: Phase II dose-ranging study design



As illustrated in Figure 7-6, the 48-hour infusion of serelaxin improved dyspnea, as assessed by both the VAS AUC and Likert endpoints. Notably, there was consistent improvement in VAS AUC across the dose groups of 10, 30 and 100 µg/kg/day reaching 2,500, 2,567 and 2,486 mm-hours, respectively, compared to 1,679 mm-hours in the placebo group. The 30 µg/kg/day dose group (40.5%) had significantly more patients with marked or moderate improvement in Likert scale compared to placebo (23.0%). The effects on the VAS AUC across doses were more consistent than the effects on the Likert scale.

Figure 7-6 Pre-RELAX-AHF: improvement in dyspnea as assessed by VAS AUC and Likert



The effects of different doses of serelaxin on an array of relevant short-term and long-term clinical endpoints in the Pre-RELAX-AHF study are summarized by dose groups in Table 7-2. Serelaxin treatment was associated with consistent trends (defined as two-sided p-values < 0.20) in relieving dyspnea and improving both the short-term and long-term clinical outcomes in patients with AHF. Although the Pre-RELAX-AHF study was not powered for statistical significance, the 30 µg/kg/day dose of serelaxin demonstrated favorable trends vs. placebo (two-sided p <0.20) on 6 of 8 clinical endpoints and showed the most consistent benefits with the largest effect size (using multi-domain analysis, Davison et al 2011). Serelaxin doses higher than 30 µg/kg/day were not associated with greater treatment effects.

As described in Section 2.3.1 and Section 7.1.2, a WHF event represents in-hospital deterioration in the clinical course of AHF and was assessed by the physicians in the Pre-RELAX-AHF study. In the Pre-RELAX-AHF study, the cumulative incidence of WHF through Day 5 was numerically lower with the active treatment doses compared to placebo. By Day 5, fewer patients in the serelaxin groups experienced WHF events compared to the placebo group (21.3% of patients in the placebo group versus 20.0%, 11.9%, 13.5%, and 10.2% in the 10, 30, 100, and 250 µg/kg/day groups respectively), suggesting a potential benefit in the improvement of this important in-hospital outcome in AHF patients (Table 7-2).

In addition, the estimated probability of CV death by Day 180 was also lower for all serelaxin groups in Pre-RELAX-AHF study, with KM estimates of 2.5%, 0.0%, 2.9%, and 6.2% in the 10, 30, 100, and 250 µg/kg/day groups respectively, compared to 14.3% in the placebo group. A post-hoc analysis using Fisher's exact two-sided test showed that the number of deaths in the 30 µg/kg/day group was significantly lower than in the placebo group (p=0.040).

Table 7-2 Pre-RELAX-AHF: effects of serelaxin on various treatment targets by dose

			Dose of serelaxin (µg/kg/day)			
Outcome	**Statistic**	**Placebo N=61**	**10 N=40**	**30 N=42**	**100 N=37**	**250 N=49**
Proportion with moderate/marked dyspnea improvement at 6, 12, & 24 h (Likert) #	proportion	23%	27.5%	40.5%	13.5%	22.4%
	p-value	-	p=0.5	p=0.037	p=0.27	p=0.88
VAS AUC change from the baseline to Day 5 (mm-hr)	mean (95% CI)	1679 (1024-2334)	2500 (1570-3430)	2567 (1664-3470)	2486 (1531-3441)	2155 (1483-2826)
	p-value	-	p=0.15	p=0.11	p=0.16	p=0.31
WHF through Day 5	proportion	21%	20%	12%	14%	10%
	p-value	-	p=0.75	p=0.29	p=0.40	p=0.15
Length of hospital stay (days)	mean (95% CI)	12.0 (10.1-13.9)	10.9 (8.1-13.6)	10.2 (8.3-12.1)	11.1 (8.9-13.3)	10.6 (8.7-12.4)
	p-value	-	p=0.36	p=0.18	p=0.75	p=0.20
Persistent renal impairment (CR increase 0.3 mg/dL or more at Days 5 & 14)	proportion	7%	8%	7%	11%	15%
	p-value	-	p=0.87	p=0.90	p=0.47	p=0.19
Days alive and out of hospital through Day 60	mean (95% CI)	44.2 (40.6-47.8)	47.0 (42.9-51.2)	47.9 (44.7-51.0)	48.0 (44.6-51.3)	47.6 (44.1-51.0)
	p-value	-	p=0.40	p=0.16	p=0.40	p=0.048
CV death or re-hospitalization due to HF or renal failure through Day 60	KM est. (%)	17.2%	10.1%	2.6%	8.4%	6.2%
	HR (95% Wald CI)	-	0.55 (0.17-1.77)	0.13 (0.02-1.03)	0.46 (0.13-1.66)	0.32 (0.09-1.17)
	p-value	-	p=0.32	p=0.053	p=0.23	p=0.085
CV mortality through Day 180			KM estimate (%)			
	Day 60	6.9%	2.5%	0.0%	2.9%	6.2%
	Day 180	14.3%	2.5%	0.0%	2.9%	6.2%
	HR (95% Wald CI)	-	0.19 (0.02-1.57)	0.00 (NA)	0.22 (0.03-1.76)	0.49 (0.13-1.90)
	Fisher's exact 2-sided test	-	p=0.14	p=0.04	p=0.15	p=0.51

P values are vs. placebo

Data shown in the dark gray boxes are those that were statistically significant versus placebo; data in light grey boxes show favorable trends, defined for the purposes of this analysis as p <0.2; data in black boxes depicts parameters that appear to be adversely affected by serelaxin (p <0.2).

#Note: In the Pre-RELAX-AHF study, the primary endpoint for the Likert score was defined as improvement at both 12 and 24 hours.

Serelaxin was generally well-tolerated, with the majority of patients completing the study on treatment. A decrease in blood pressure was more frequently reported in the serelaxin groups than the placebo group. These events were clinically manageable, and there was no evidence of a dose-response relation. The 250 µg/kg/day dose of IV serelaxin was associated with numerically higher rate of worsening renal function, defined as persistent increase in creatinine from baseline ≥0.3 mg/dL at Days 5 and 14 (Table 7-2).

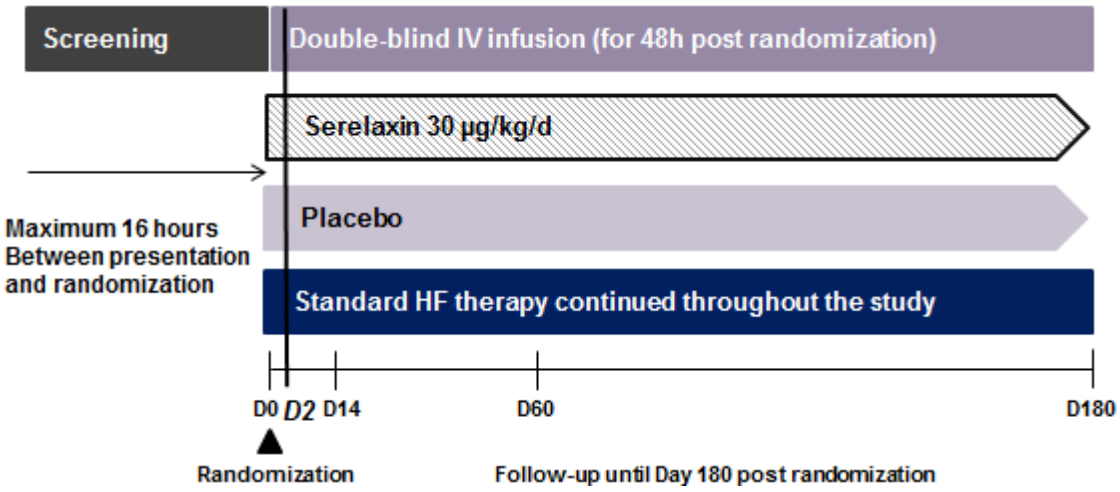
The efficacy and safety results summarized above supported the selection of 30 µg/kg/day dose for further study. Hence, the 48-hour infusion of serelaxin dose at 30 µg/kg/day was selected for testing in the Phase III pivotal study RELAX-AHF.

7.3 Phase III Pivotal Study: RELAX-AHF

7.3.1 Study design

The design of the Phase III pivotal RELAX-AHF study is described in Figure 7-7. It was discussed with the FDA at the end of Phase II, and advice received from the health authorities was considered in the design/conduct of the Phase III program.

Figure 7-7 RELAX-AHF study design



The key features considered for the Phase III pivotal study design are described below.

7.3.1.1 Study endpoints

Primary endpoints:

The primary efficacy assessments of changes in dyspnea in the RELAX-AHF study were:

- Area under the curve (AUC) representing the absolute change in patient-reported dyspnea from baseline through Day 5 measured by 100-mm VAS;
- Moderately or markedly improved dyspnea relative to the start of study drug infusion at 6, 12 and 24 hours on Day 1 (required at all 3 time points) measured by 7-point Likert scale.

VAS was administered to randomized patients at baseline, 6, 12 and 24 hours, and daily through Day 5 followed by final assessment at Day 14. The Likert scale was also administered at the same fixed timepoints.

The VAS AUC assessed both improvement and worsening of dyspnea through Day 5, whereas the Likert scale assessed only moderate or marked improvement during the first 24 hours (Section 7.1.1).

Criteria for a positive pivotal study: Based on criteria pre-specified in the study protocol and statistical analysis plan with the Type I error rate for global null hypothesis controlled for comparisons between the two primary endpoints at the two-sided 0.05 level (Hochberg approach), the study was considered to have met the primary study objective of demonstrating efficacy of serelaxin in dyspnea relief if either primary endpoint was statistically significant at the two-sided 0.025 level (alpha was evenly split), or if both tests were significant at the two-sided 0.05 level.

Secondary endpoints:

The two secondary efficacy endpoints for the Phase III RELAX-AHF study were:

- CV death or re-hospitalization due to HF or renal failure through Day 60
- Days alive and out of hospital (DAOOH) through Day 60

Additional efficacy endpoints:

In addition to its inclusion into the VAS AUC primary endpoint, WHF as an endpoint was defined as a composite of an in-hospital WHF event, rehospitalization for HF, or death up to Day 5 or 14, respectively.

Other pre-specified in-hospital efficacy endpoints included physician's assessments of the signs and symptoms of congestion, doses of IV loop diuretics, and length of hospital stay including days in intensive care unit (ICU)/coronary care unit (CCU).

Mortality

CV mortality through Day 180 was pre-specified as an additional efficacy endpoint, and all-cause mortality through Day 180 was pre-specified as a safety endpoint.

Biomarkers

Three key biomarkers, cardiac troponin T measured with a high sensitivity assay (hs-cTnT), NT-proBNP and cystatin-C were measured at baseline and at various time points (Day 2, Day 5 and Day 14) during the follow-up period. In addition, cystatin-C was also measured at Day 60. These biomarkers were pre-specified in the report analysis plan as indicators of myocardial injury (hs-cTnT), kidney function (cystatin-C) and cardiac wall stress (NT-proBNP).

Safety reporting

The non-serious AEs and serious adverse events (SAEs) were to be reported through Day 5 and Day 14, respectively, in the study.

7.3.1.2 Key patient eligibility criteria

Patients enrolled in RELAX-AHF study were hospitalized for AHF and had to meet the following key inclusion criteria:

1. Hospitalized for AHF, with AHF defined as including all of the following measured at any time between presentation (including the emergency department [ED]) and the end of screening:
   - Dyspnea at rest or with minimal exertion
   - Pulmonary congestion on chest radiograph
   - BNP ≥350pg/mL or NT-proBNP ≥1400 pg/mL
2. Systolic blood pressure >125 mmHg at the start and end of screening
3. Able to be randomized within 16 hours from presentation to the hospital, including the ED
4. Received IV furosemide of at least 40 mg (or equivalent) at any time between admission to emergency services (either ambulance or hospital, including the ED) and the start of screening for the study
5. Impaired renal function defined as eGFR (sMDRD calculation) on admission between 30-75 mL/min/1.73 m^2

7.3.1.3 Dosing of study drug

In the Phase III RELAX-AHF study, serelaxin dosing was based on body weight and was administered over 48 hours at a constant infusion rate of 10 mL/hour with two consecutive infusions each of 24-hour duration. The volume of serelaxin at the tested dose of 30 µg/kg/day required to prepare each 24-hour infusion was calculated per body weight category as shown in Table 7-3.

Table 7-3 Dosing of serelaxin per body weight category – RELAX-AHF

Actual body weight (kg)	Serelaxin (mg)	Volume of serelaxin to be added to 250 mL of sterile 5% (w/v) dextrose for infusion over 24 hours (mL)
40−59	2.0	2.0
60−74	3.0	3.0
75−114	3.5	3.5
115−160	5.5	5.5

In addition, during the 48-hour infusion period, if a patient's SBP dropped by >40 mmHg from pre-treatment values but remained ≥100 mmHg, or fell to <100 mmHg, the protocol-required dose adjustments outlined in Table 7-4 were implemented.

Table 7-4 Serelaxin dose adjustments required during infusion in the event of reduction in systolic blood pressure – RELAX-AHF

Systolic blood pressure during infusion*	Adjustments in serelaxin treatment
Decreased by >40 mmHg from pre-treatment but remains ≥100 mmHg	Decrease the infusion rate by 50% for the remainder of the 48-hour infusion (i.e., from 10 mL/h to 5 mL/h)
<100 mmHg	Discontinue the infusion

* Blood pressure values were confirmed by two measurements taken 15 minutes apart.

7.3.1.4 DMC/CEC process

An independent external Data Monitoring Committee (DMC) monitored patient safety and efficacy of serelaxin in AHF patients who participated in the RELAX-AHF study. A central Clinical Event Committee (CEC) adjudicated all deaths and re-hospitalizations (except for planned or elective admissions) that occurred through Day 60. In addition, a post-hoc adjudication of the deaths that occurred between Day 61 and Day 180 in RELAX-AHF study was performed by the same CEC while still blinded to the study results.

7.3.1.5 Statistical methodology for efficacy analysis and study sample size

7.3.1.5.1 Efficacy analysis

Primary efficacy analysis

The main efficacy analysis presented from the Phase III RELAX-AHF study utilized the intent-to-treat (ITT) set, which included all randomized subjects, whether or not they were exposed to study drug.

The primary analysis of AUC of VAS change from baseline was performed using a t-test. The t-test was the pre-specified primary analysis method for the AUC of VAS change from baseline and was the basis for the interpretation of the primary VAS endpoint results. Asymptotically (for large sample sizes such as in RELAX-AHF), the t-test statistic is normally distributed. Therefore, the t-test remains valid even when individual patient data depart from normality (Rasch and Guiard 2004).

A pre-specified supportive analysis using the Wilcoxon rank-sum test was also performed in the event the data did not follow a normal distribution, i.e. the test of non-normality was significant ($P<0.005$ in either treatment group).

For Likert data, the proportion of patients with moderately or markedly better dyspnea at 6, 12, and 24 hours (all three time points) was compared between treatment groups using a chi-square test.

For all subgroup analyses on primary endpoint, the subgroup variable and interaction term between treatments and subgroup variables were also included in the model.

Data assignment for death and WHF events

The rationale for the pre-specified dyspnea score assignment for an event of death or WHF is described in detail in Section 7.1.2. VAS and Likert measurements for the primary efficacy analysis as well as investigator's assessments of HF signs and symptoms were analyzed as follows:

- For patients who died or had an in-hospital WHF event, the worst score observed for any patient at any time-point in the study was assigned for all time-points after the onset of WHF event or death whichever occurred earlier, regardless of whether the score was missing or not.
- Other missing data was imputed using linear interpolation or last observation carry-forward (LOCF). If there was no final measurement, then LOCF was used.

Other efficacy analyses

The time to WHF through Day 5, length of hospital stay, and DAOOH were analyzed using a Wilcoxon rank sum test. In addition, endpoints such as mortality and composite of CV death and rehospitalization were analyzed using standard time-to-event analysis methods, using log-rank tests for treatment comparisons and Cox proportional hazard models with treatment as a factor for estimation of hazard ratios.

Log-transformed biomarker data were analyzed using repeated measures analysis of change from baseline. Exploratory analyses were performed to examine the relationship between the 'change in baseline to Day 2 of biomarkers' and 'mortality at Day 180' using Cox regression with the change from baseline on Day 2 biomarker category as the factor in the model.

Subgroup analyses were performed for the RELAX-AHF study for primary endpoints. A number of post-hoc subgroup analyses were also performed to address health authority requests (Section 7.3.3.5).

Finally, the Cox proportional hazards regression model was used to evaluate the association between the occurrence of in-hospital WHF events through Day 5 and longer term outcomes such as mortality through Day 180. In this analysis, patients who died prior to Day 5 were excluded.

7.3.1.5.2 Study sample size

The study was designed to test the two primary hypotheses at the two-sided 0.05 significance level with adjustment for multiplicity using the Hochberg approach. The estimated sample size of RELAX-AHF study was 1,160 patients, which provided ~80% study power to detect clinically relevant effects of serelaxin compared to placebo for the primary endpoints, i.e., a treatment difference of 468 mm-hour (4 mm on average) in the VAS AUC endpoint, and a relative risk of 1.3 (absolute difference of 7.5%) in the Likert scale endpoint.

7.3.2 Patient disposition and baseline patient characteristics

7.3.2.1 Patient disposition

Patient disposition is summarized in Table 7-5. A total of 1,161 patients were randomized to receive either serelaxin (581 patients) or placebo (580 patients). A total of 1,035 patients (89.1%) completed the study as planned after taking serelaxin (530 patients, 91.2%) or placebo (505 patients, 87.1%). The remaining 126 patients (10.9%) who did not complete Day 180 either died (106 patients, 9.1%), withdrew consent from follow-up (18 patients, 1.6%), or were lost to follow-up (2 patients 0.2%). The proportions of patients who either completed the study or withdrew consent were similar for the serelaxin and placebo treatment groups. The vital status at 180 days could not be ascertained in 14 patients (12 patients withdrew from follow-up and 2 were lost to follow-up; 1.2%) with equal distribution between the serelaxin (n=7) and placebo (n=7) groups.

Table 7-5 Patient disposition - RELAX-AHF (ITT set)

	Placebo N=580 (%)	Serelaxin N=581 (%)
Completed Day 180		
Yes	505 (87.1)	530 (91.2)
No	75 (12.9)	51 (8.8)
Died	65 (11.2)	41[3] (7.1)
Withdrew consent from follow-up [1, 2]	9 (1.6)	9 (1.5)
Lost to follow-up	1 (0.2)	1 (0.2)

[1] Patients who withdrew consent from follow-up are patients who both withdrew consent from contact and withdrew consent for access to hospital records.

[2] Vital status was determined for 6 of these 18 patients through public records or regular office visits as permitted by local regulations.

[3] One patient, who withdrew consent, died on Day 104 and is therefore not included as withdrawn due to death in the disposition table.

7.3.2.2 Baseline demographic and disease characteristics

The demographic characteristics in the two treatment groups were similar (Table 7-6). The mean age was ~72.0 years with over 75% patients ≥65 years old. The majority of patients were white (94.4%) and almost two thirds were male (62.4%).

Table 7-6 Baseline patient demographic characteristics - RELAX-AHF (ITT set)

Parameter	Statistic	Placebo (N=580)	Serelaxin (N=581)
Age (years)	n'	580	581
	Mean (SD)	72.5 (10.78)	71.6 (11.68)
	Median	74	73
	Min, Max	24, 96	29, 97
	<65	119 (20.5)	145 (25.0)
	≥65	461 (79.5)	436 (75.0)
	<75	296 (51.0)	315 (54.2)
	≥75	284 (49.0)	266 (45.8)
Gender – n (%)	Male	357 (61.6)	368 (63.3)
	Female	223 (38.4)	213 (36.7)
Ethnicity – n (%)	Not Hispanic or Latino	521 (89.8)	523 (90.2)
	Hispanic or Latino	59 (10.2)	57 (9.8)
Race – n (%)	American Indian or Alaska Native	0 (0.0)	1 (0.2)
	Asian	2 (0.3)	2 (0.3)
	Black or African American	23 (4.0)	29 (5.0)
	Native Hawaiian or Pacific Islander	0 (0.0)	1 (0.2)
	White	552 (95.2)	544 (93.6)
	Multi-Racial	1 (0.2)	1 (0.2)
	Other	2 (0.3)	3 (0.5)
Weight (kg)	n'	579	580
	Mean (SD)	82.8 (18.70)	81.9 (18.52)
	Median	80.5	80.2
	Min, Max	40.3, 158.3	40.8, 148.1
Height (cm)	n'	570	573
	Mean (SD)	167.3 (9.54)	167.4 (9.51)
	Median	168	168
	Min, Max	140.0, 198.0	132.0, 193.0

The baseline disease characteristics by treatment group are summarized in Table 7-7. Mean SBP was similar (142 mmHg) for both the serelaxin and placebo groups. As expected, patients in both treatment groups presented with a high but similar frequency of cardiovascular and metabolic comorbidities, such as hypertension, ischemic heart disease, atrial fibrillation/flutter, hyperlipidemia, and diabetes mellitus. Patients were randomized into the study from presentation within an average of 8 hours for both treatment groups.

The proportion of patients who had a confirmed history of CHF one month prior to index admission was similar in the serelaxin and placebo groups (75.2% vs. 73.1%). In addition, the proportion of patients with a most recent ejection fraction (EF) <40% was higher than the proportion of patients with EF ≥40% (54.8% vs. 45.2%) and was similar in both treatment groups. A greater proportion of serelaxin-treated patients were hospitalized for HF (1 or more hospitalizations) within the previous year than placebo patients (37.2% vs. 31.2%, p=0.0354).

Table 7-7 Patient baseline disease characteristics - RELAX-AHF (ITT set)

Baseline characteristic	Statistic	Placebo (N=580)	Serelaxin (N=581)
SBP at baseline (mmHg)	n'	578	577
	Mean	142.12	142.20
	(SD)	(16.994)	(16.196)
	Median	140	139
<130 mmHg	n (%)	122 (21.1)	107 (18.5)
130 to <140 mmHg	n (%)	162 (28.0)	191 (33.1)
140 to <152 mmHg	n (%)	153 (26.5)	138 (23.9)
≥152 mmHg	n (%)	141 (24.4)	141 (24.4)
Hospitalized for HF in past year	n (%)	181 (31.2)	216 (37.2)
Number of HF hospitalizations in past year	n'	180	214
	Mean (SD)	1.5 (1.13)	1.7 (1.48)
	Median	1	1
	Min, Max	1, 9	1, 11
Days since discharge from last hospitalization	n'	156	196
	Mean	125.2	118.1
	(SD)	(123.62)	(112.81)
	Median	85	86.5
	Min, Max	2, 883	2, 657
Medical history		n (%)	n (%)
Hypertension		510 (87.9)	496 (85.4)
Hyperlipidemia		313 (54.0)	304 (52.3)
Stroke/other cerebrovascular event		84 (14.5)	73 (12.6)
Substance abuse		15 (2.6)	16 (2.8)
Cigarette smoking		81 (14.0)	72 (12.4)
Depression		31 (5.3)	29 (5.0)
Hyperthyroid		21 (3.6)	14 (2.4)
Hypothyroid		40 (6.9)	42 (7.2)
Peripheral vascular disease		82 (14.1)	73 (12.6)
Malignancy		38 (6.6)	41 (7.1)
Mitral stenosis		6 (1.0)	8 (1.4)
Mitral regurgitation		182 (31.4)	179 (30.8)
Aortic stenosis		28 (4.8)	25 (4.3)
Aortic regurgitation		26 (4.5)	41 (7.1)
Pacemaker		58 (10.0)	63 (10.8)
Biventricular pacing		52 (9.0)	61 (10.5)

Baseline characteristic	Statistic	Placebo (N=580)	Serelaxin (N=581)
Auto. internal cardiac defibrillator		75 (12.9)	79 (13.6)
Asthma, bronchitis, or COPD		88 (15.2)	96 (16.5)
Ischemic heart disease		307 (52.9)	296 (50.9)
Myocardial Infarction		202 (65.8)	201 (67.9)
Coronary artery bypass graft		112 (36.5)	99 (33.4)
Percutaneous intervention		140 (45.6)	150 (50.7)
Angina pectoris		66 (11.4)	72 (12.4)
Atrial fibrillation/flutter		305 (52.6)	297 (51.1)
Chronic		186 (61.0)	164 (55.2)
Paroxysmal		72 (23.6)	78 (26.3)
Persistent		47 (15.4)	55 (18.5)
Diabetes mellitus		272 (46.9)	279 (48.0)
Controlled by insulin		111 (40.8)	117 (41.9)
Controlled by oral antidiabetic agents		147 (54.0)	171 (61.3)
Controlled by diet only		36 (13.2)	25 (9.0)
Concomitant medications		n (%)	n (%)
ACE inhibitors		320 (55.2)	313 (53.9)
Angiotension receptor blockers		97 (16.7)	88 (15.1)
Beta-blockers		407 (70.2)	387 (66.6)
Aldosterone antagonists		173 (29.8)	193 (33.2)
Digoxin		108 (18.6)	120 (20.7)
IV nitrates at randomization		42 (7.2)	39 (6.7)
CHF 1 month prior to admission	n (%)	424 (73.1)	437 (75.2)
New York Heart Association (NYHA)			
I	n (%)	11 (2.6)	12 (2.7)
II	n (%)	140 (33.0)	164 (37.5)
III	n (%)	198 (46.7)	191 (43.7)
IV	n (%)	72 (17.0)	63 (14.4)
Time - presentation to randomization	Mean (h)	7.9	7.8
Most recent ejection fraction	n'	539	552
<30%	n (%)	131 (24.3)	152 (27.5)
≥30%	n (%)	408 (75.7)	400 (72.5)
<35%	n (%)	216 (40.1)	234 (42.4)
≥35%	n (%)	323 (59.9)	318 (57.6)
<40%	n (%)	295 (54.7)	303 (54.9)
≥40%	n (%)	244 (45.3)	249 (45.1)
eGFR at baseline	n'	580	580
	Mean	53.28	53.71
	(SD)	(12.937)	(13.131)
	Median	52.95	53.10

Baseline characteristic	Statistic	Placebo (N=580)	Serelaxin (N=581)
	Min, Max	17.0, 116.6	25.4, 106.4
hs-Troponin T (µg/L)			
	n'	541	533
	Geometric mean	0.0361	0.0341
	95% CI	0.0336, 0.0388	0.0318, 0.0366
	Median	0.034	0.033
	Range	0.007–1.890	0.007–1.110
	Below 99th percentile	49 (9.1%)	57 (10.7%)
NT-pro-BNP levels (pg/mL)			
	n'	551	550
	Geometric mean	5003.50	5125.46
	95% CI	4633.05, 5403.57	4771.58, 5505.58
	Median	4942.0	4758.0
	Range	96.0–552500.0	239.0-52500.0
Cystatin-C levels (mg/L)			
	n'	551	550
	Geometric mean	1.46	1.45
	95% CI	1.42, 1.50	1.42, 1.49
	Median	1.5	1.5
	Range	0.3, 4.5	0.6, 3.5

n' patients with measurements. Note biomarker values were collected for a subset of patients.

These major baseline demographic and disease characteristics of the AHF patients randomized into RELAX-AHF were similar to those described in contemporary AHF patient registries (Table 7-8), suggesting that the studied patient population in RELAX-AHF represented the real-world AHF patient population.

Finally, most baseline biomarker levels were also similar in both two treatment groups. At baseline, approximately 90% of patients in both treatment groups had hs-cTnT levels above the 99th percentile, reflecting the occurrence of myocardial injury during AHF.

Table 7-8 Comparison of key baseline characteristics of AHF patients in RELAX-AHF with patient registries

Baseline characteristic	RELAX-AHF (N=1,161)	ADHERE [2] (N=105,388)	OPTIMIZE [1] (N=34,059)
Mean age (years)	72	72	73
Women (%)	37	52	52
Baseline SBP (mmHg)	142	144	143
Prior HF (%)	74	75	87
LVEF <40% (%)	55	54	52
eGFR <60 mL/min (%)	72	64	NA
Hypertension (%)	87	73	71
Coronary artery disease (%)	52	57	50
Diabetes (%)	48	44	42
Atrial fibrillation (%)	52	31	31

[1] Gheorghiade et al 2005

[2] Adams et al 2005

Standard-of-care HF treatments in RELAX-AHF

The use of concomitant HF medications (ACE inhibitors, ARBs, aldosterone antagonists and β-blockers) was similar at baseline in both serelaxin and placebo groups (Table 7-7). After randomization, patients in RELAX-AHF received guideline-recommended HF therapies throughout the study period, such as ACE inhibitors (60% in serelaxin vs. 57% in placebo), ARBs (17% vs. 21%), aldosterone antagonist (45% vs. 49%), and β-blockers (76% vs. 79%), reported at Day 60.

The use of IV nitrates at baseline prior to randomization was reported in 6.7% and 7.2% of serelaxin and placebo groups respectively (Table 7-7), while during the study drug infusion, 9.5% of serelaxin and 12.4% of placebo patients received IV nitrates.

7.3.3 Primary efficacy outcomes

7.3.3.1 Primary efficacy results on VAS

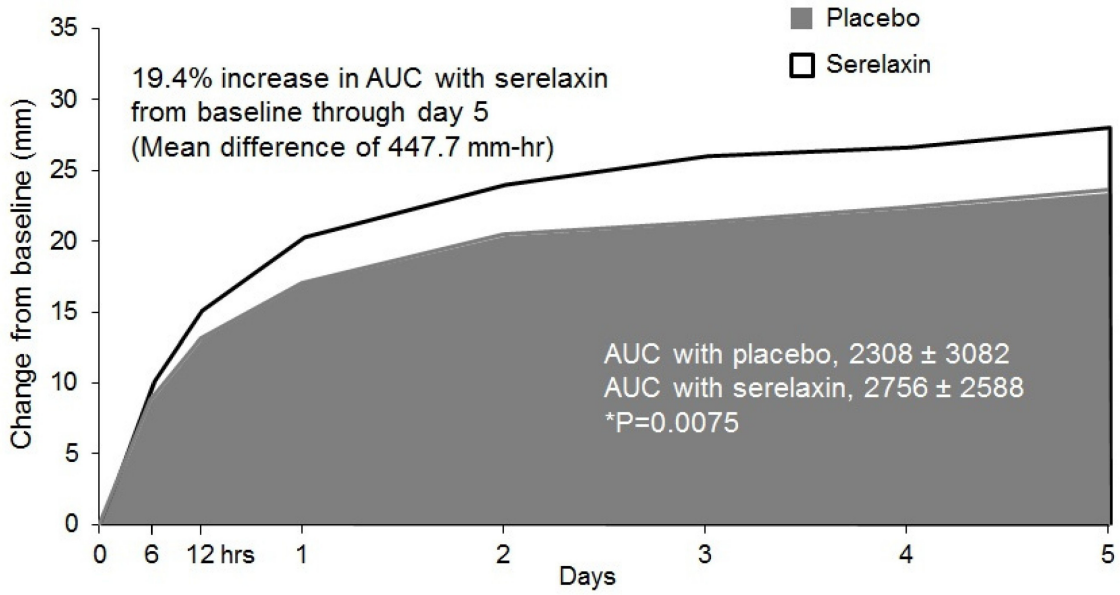
Baseline VAS scores were similar in both the serelaxin and placebo groups, with a mean value of 44.2 mm (tertile ranges: lower ≤35 mm; middle >35 to 52 mm; upper >52 mm) reported in the total patient population. It should be noted, however, that the VAS scores reported at baseline likely underestimated the severity of dyspnea at the time of initial presentation since there was an average delay of approximately 8 hours from the time of initial patient presentation at hospital to randomization into the study (Table 7-7). During this time, patients received treatment for their AHF episodes.

Primary analysis

The VAS AUC primary endpoint reflects both patient improvement and worsening of dyspnea. There was a statistically significant improvement in the VAS AUC from baseline through Day 5 in the serelaxin-treated patients. In the ITT analysis set, the mean change in VAS AUC Day 0-5 in the serelaxin and placebo groups was 2,756 mm-hours and 2,308 mm-hours, respectively. The mean treatment difference was 447.7 mm-hours (95% CI: 120.0 to 775.4 mm-hours; p=0.0075) representing a 19.4% relative increase in the VAS AUC over the observed placebo response (Figure 7-8) (Table 7-9). In addition, when the analysis was adjusted for baseline VAS decile, age, gender, race and country (as pre-specified in the statistical analysis plan), the significant treatment difference was maintained (p=0.00196).

It is important to note that the VAS AUC improvement from baseline through Day 5 seen in RELAX-AHF was accompanied by a significantly lower total dose of IV loop diuretics in serelaxin-treated patients (Section 7.3.7).

Figure 7-8 Mean change from baseline (mm) of VAS - RELAX-AHF (ITT set)



* P-value is based on a two-sided two sample t-test for serelaxin versus placebo comparing Mean AUC (mm-hours) of change from baseline of dyspnea VAS through Day 5

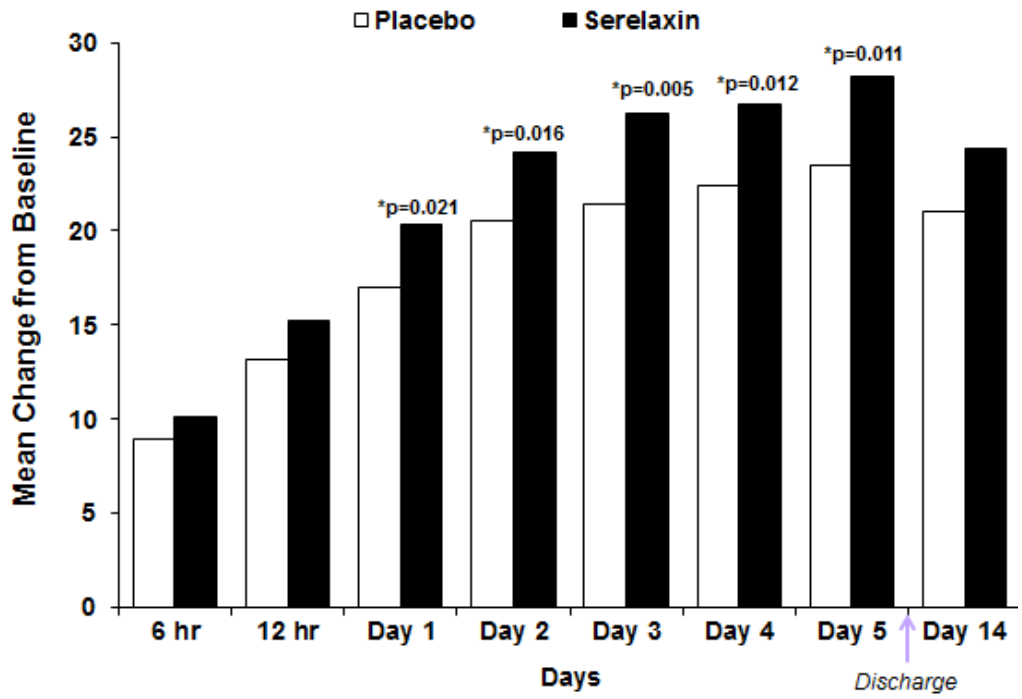
Table 7-9 **Area under the curve (AUC, mm-hours) of change from baseline of dyspnea Visual Analog Scale (VAS) through Day 5 - RELAX-AHF (ITT set)**

	AUC Baseline/Day 0 to Day 5	
Statistic	**Placebo (N=580)**	**Serelaxin (N=581)**
n'	580	581
Mean (SD)	2308 (3082)	2756 (2588)
95% CI	2057, 2559	2545, 2966
Median	2436	2742
Q1, Q3	1097, 4086	1170, 4239
Min, Max	-11040, 10698	-7137, 11115
Mean difference		447.7
95% CI		120.0, 775.4
p-value [1]		0.0075

[1] p-value is based on a two-sided two sample t-test for serelaxin versus placebo comparing AUC (mm-hours) of change from baseline of dyspnea visual analog scale (VAS) through Day 5

As illustrated in Figure 7-9, serelaxin treatment was associated with a significantly better VAS score than placebo at all time points beyond 6 hours. Notable improvements ($p < 0.05$, t-test) in dyspnea VAS scores for serelaxin compared to placebo were seen at 24 hours (p=0.021), 48 hours (p=0.016), Day 3 (p=0.005), Day 4 (p=0.012), and Day 5 (p=0.011).

Figure 7-9 **Mean change from baseline (mm) of VAS by time points - RELAX-AHF (ITT set)**



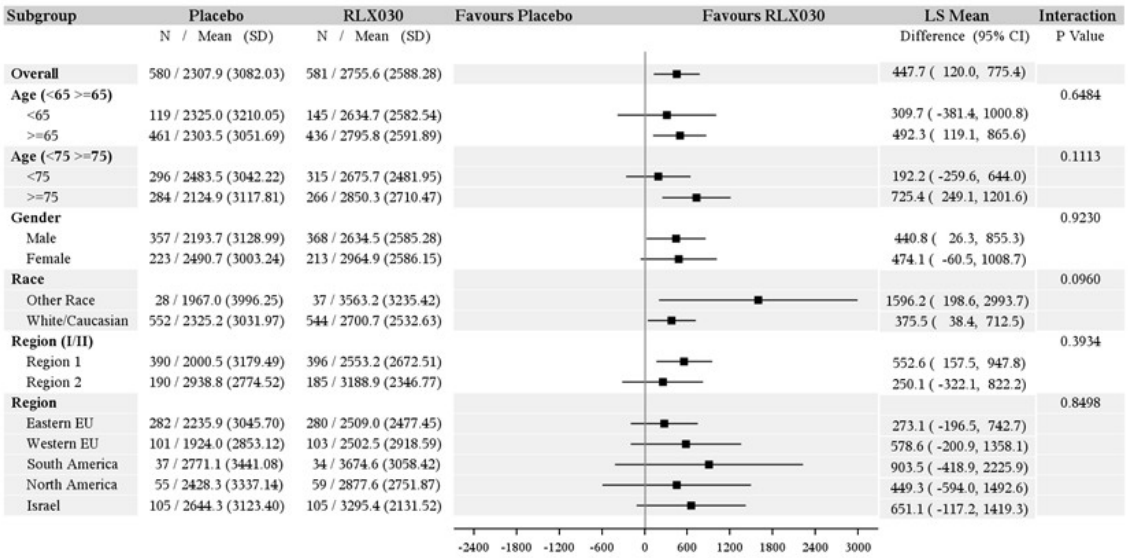
* p-values by t-test are shown for time points where p<0.05. P values not shown are >0.05.

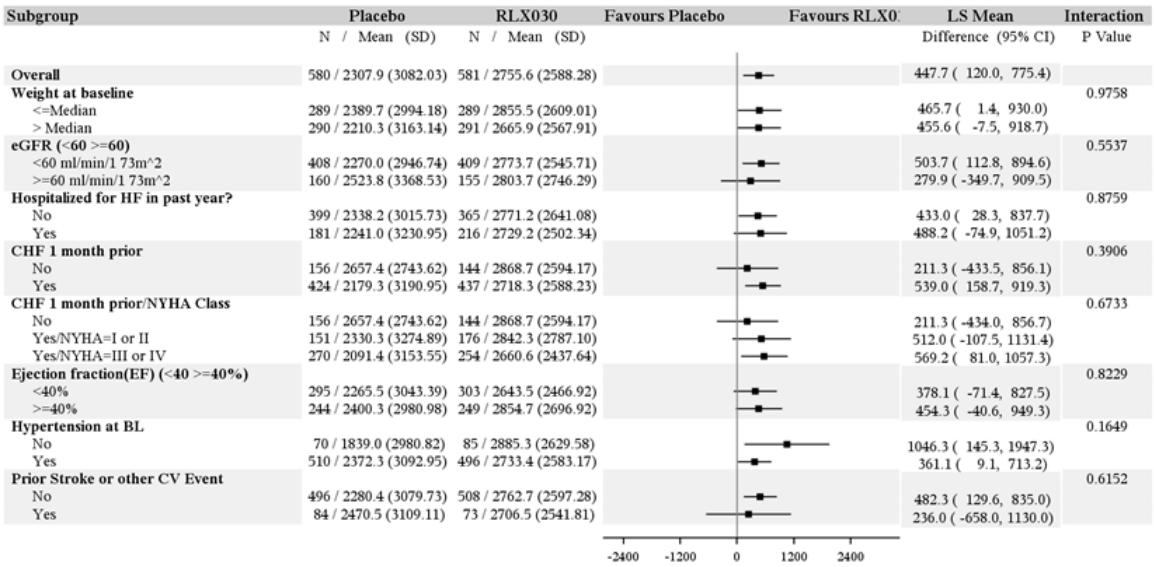
In addition, the mean VAS AUC from baseline through Day 14 was 8,442 mm-hours and 7,131 mm-hours in the serelaxin and placebo groups, respectively, and the treatment difference of 1,311 mm-hours was also statistically significant based on t-test (p=0.017).

Subgroup analysis

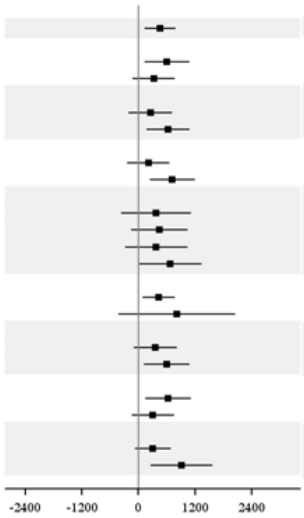
The forest plots shown in Figure 7-10 summarize the treatment differences for VAS AUC of the mean change from baseline through Day 5 by demographic and clinical subgroups. Mean treatment differences were numerically in favor of serelaxin compared with placebo across the subgroups, and there were no statistically significant treatment by subgroup interactions.

Figure 7-10 VAS AUC of mean change from baseline (mm-hr) by demographic and clinical subgroups in RELAX-AHF (ITT set)





Subgroup	Placebo N / Mean (SD)	RLX030 N / Mean (SD)	LS Mean Difference (95% CI)	Interaction P Value
Overall	580 / 2307.9 (3082.03)	581 / 2755.6 (2588.28)	447.7 (120.0, 775.4)	
Ischemic heart disease				0.3973
No	265 / 2238.6 (2995.64)	276 / 2837.6 (2503.84)	599.0 (118.5, 1079.4)	
Yes	315 / 2366.2 (3156.44)	305 / 2681.4 (2664.31)	315.2 (-133.5, 764.0)	
Atrial Fibrillation/Flutter at BL				0.2588
No	275 / 2754.4 (2887.78)	284 / 2997.8 (2512.55)	243.4 (-225.9, 712.7)	
Yes	305 / 1905.3 (3198.81)	297 / 2524.0 (2642.15)	618.7 (166.5, 1070.9)	
Diabetes Mellitus				0.1279
No	308 / 2327.5 (2963.15)	302 / 2531.2 (2576.32)	203.6 (-248.1, 655.4)	
Yes	272 / 2285.7 (3216.70)	279 / 2998.5 (2583.83)	712.9 (237.5, 1188.2)	
Systolic BP at BL				0.9143
<130 mmHg	122 / 2003.5 (3332.98)	107 / 2366.5 (2617.88)	363.0 (-376.7, 1102.7)	
130-<140 mmHg	162 / 2359.5 (2773.15)	191 / 2797.0 (2552.19)	437.5 (-159.1, 1034.0)	
140-<152 mmHg	153 / 2553.2 (2517.21)	138 / 2924.4 (2627.59)	371.2 (-284.5, 1026.8)	
>=152 mmHg	141 / 2239.2 (3700.37)	141 / 2907.8 (2565.57)	668.6 (3.5, 1333.8)	
IV Nitrates Use at BL				0.5562
No	538 / 2315.6 (3031.74)	542 / 2736.8 (2503.56)	421.2 (81.2, 761.2)	
Yes	42 / 2209.8 (3707.65)	39 / 3017.5 (3597.38)	807.7 (-434.8, 2050.1)	
Heart rate at Baseline (bpm)				0.4667
<80 bpm	296 / 2496.3 (3106.22)	312 / 2845.0 (2553.88)	348.7 (-104.4, 801.7)	
>=80 bpm	282 / 2106.8 (3051.04)	263 / 2700.0 (2632.42)	593.2 (114.6, 1071.9)	
Time from Presentation to Randomization				0.3398
<6 Hr	266 / 2177.7 (2909.91)	275 / 2797.4 (2586.53)	619.7 (139.4, 1100.0)	
>=6 Hr	314 / 2418.2 (3221.15)	306 / 2718.1 (2593.50)	299.8 (-148.8, 748.5)	
History of CRT or ICD				0.1124
No	439 / 2432.1 (2924.39)	428 / 2729.4 (2628.49)	297.3 (-81.7, 676.3)	
Yes	141 / 1921.2 (3511.42)	153 / 2828.8 (2479.25)	907.6 (256.2, 1558.9)	



Note: the treatment comparison RLX030 (serelaxin) versus placebo is displayed. Mean treatment difference and p-value for interaction are from ANCOVA model with treatment, subgroup, and treatment*subgroup interaction as covariates.

Region 1 = United States, Israel, France, Germany, Italy, Spain, Poland, Netherlands

Region 2 = Argentina, Romania, Hungary

7.3.3.2 Primary efficacy results on Likert

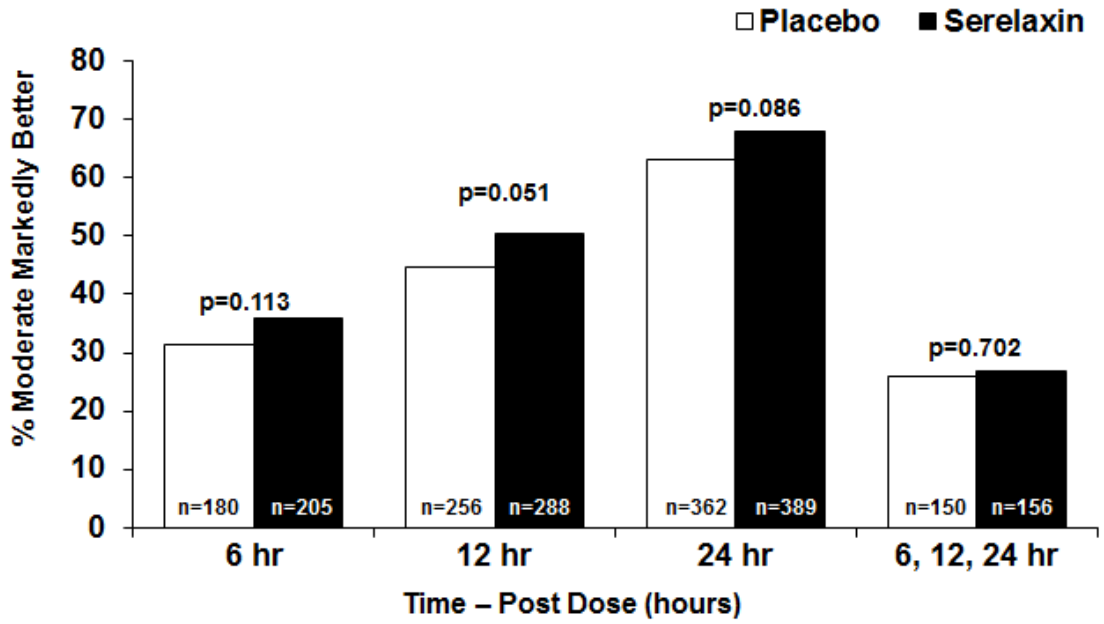
Primary analysis

In the RELAX-AHF study, the second primary efficacy endpoint was patient-reported moderate or marked dyspnea improvement relative to the start of study drug on the 7-point Likert scale at combined time points of 6, 12 and 24 hours.

In the ITT analysis set, the proportion of patients meeting this primary efficacy endpoint was 26.9% and 25.9% in the serelaxin and placebo groups respectively. The odds ratio (1.05; 95% CI: 0.81, 1.37) as an estimate of treatment effect showed that the treatment with serelaxin was not associated with a statistically significant dyspnea improvement as the endpoint was defined (p=0.7024). However there was a numerically higher proportion of patients in favor of serelaxin at the individual time points of 6 hours (35.8% vs. 31.4%), 12 hours (50.3% vs. 44.6%), and 24 hours (67.9% vs. 63.1%) (Figure 7-11).

In a pre-specified analysis of the Likert data, the time to moderate or marked improvement of dyspnea was significantly shorter for patients treated with serelaxin (mean 1.53 days) compared to placebo (mean 1.91 days) (p=0.00186; Wilcoxon rank sum test).

Figure 7-11 Moderately or markedly better dyspnea by Likert scale by time point post treatment - RELAX-AHF (ITT set)



* P-value is based on Chi-Square test for serelaxin vs. placebo; comparison at 6, 12 and 24 hours was one of two primary endpoints.

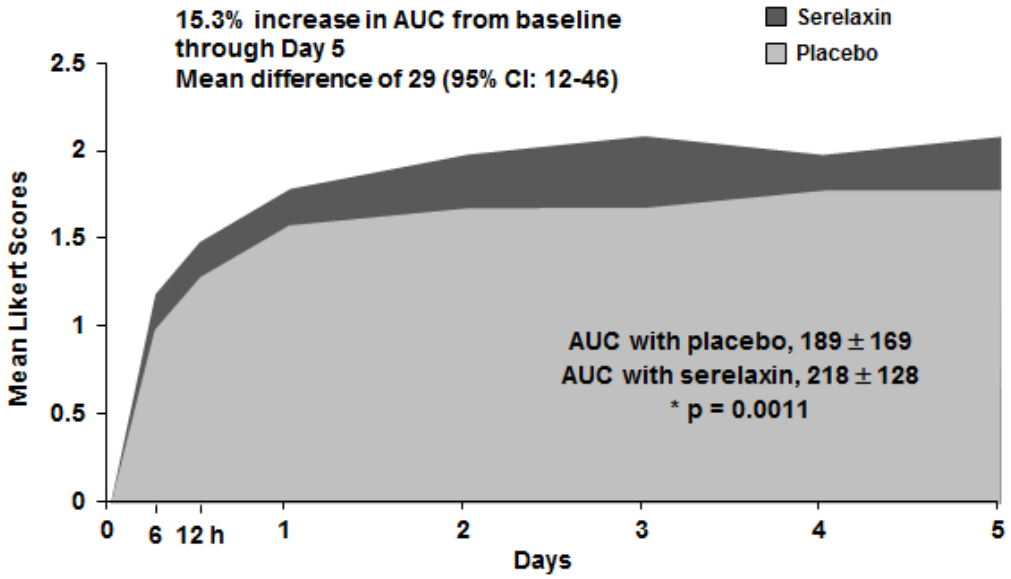
Additional analysis

The following post-hoc analyses were conducted to further understand the discordant outcomes between the VAS AUC and Likert endpoints:

- The Likert scale was reanalyzed utilizing the full 7-point scale through Day 5 (Figure 7-12) that allowed patients to be classified as worse in addition to improved. This analysis mirrors the VAS AUC analysis in both the dependent variable (AUC) and time frame (through Day 5). Although there is no baseline value for the Likert scale, each option provided on the 7-point scale asks the patient directly about how their current status is different from the baseline state. Therefore, each Likert point reflects change from baseline. Similar to the VAS AUC primary efficacy analysis, the average Likert AUC Day 0-5 was significantly greater for the serelaxin group (mean=217.7, SD=127.9) with a 15.3% relative increase compared to the placebo group (mean=188.8, SD=169.0) (p=0.0011, t-test). When restricting the AUC calculation of Likert scores to the period of first 24 hours, the mean Likert AUC was also statistically significantly greater in favor of the serelaxin group (p=0.0102).
- Moreover, when the proportions of patients reporting moderately or markedly worsening of dyspnea on the Likert scale by timepoints were compared, serelaxin treatment resulted in significantly fewer patients experiencing moderately or markedly worsening of dyspnea at all measured time points through Day 5, compared to placebo (Figure 7-13).

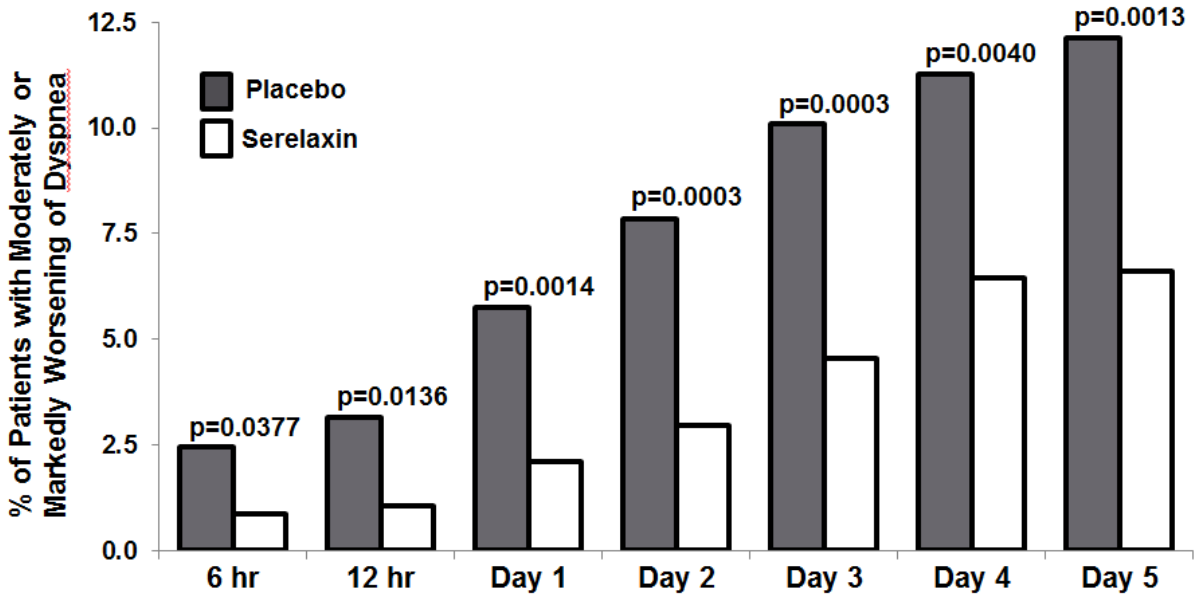
It should be noted that the pre-specified dyspnea score assignment (Section 7.1.2.2) was applied in the above two post-hoc analyses to capture the occurrence of in-hospital WHF events.

Figure 7-12 Likert AUC analysis through Day 5 - Study RELAX-AHF (ITT set)



* P-value is based on a two-sided two sample t-test for serelaxin versus placebo comparing area under the curve (AUC, unit-hours) of dyspnea Likert scale from baseline to Day 5

Figure 7-13 Cumulative proportion of patients with moderately or markedly worse dyspnea on the Likert scale



Discussion of the primary efficacy outcomes

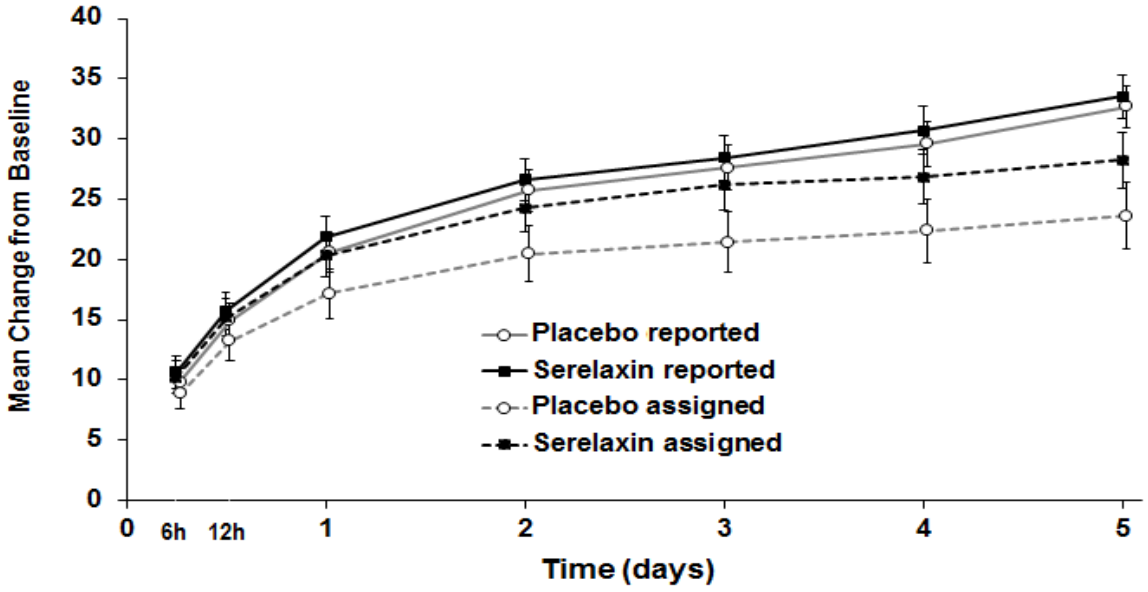
Based on the positive primary efficacy result on VAS AUC change from baseline through Day 5 (19.4% increase vs. placebo, p=0.0075), the RELAX-AHF study met its primary objective as specified in the protocol ($p<0.025$ for either primary endpoint or $p<0.05$ for both primary endpoints), demonstrating a statistically significant improvement of dyspnea in the serelaxin group compared to the placebo group.

The study did not meet the second primary efficacy endpoint based on Likert assessment of dyspnea. This was related to the fact that the VAS AUC captured both the improvement and worsening of symptoms, while the Likert primary endpoint as pre-specified in the RELAX-AHF study protocol, reflected only moderate or marked patient improvement. This is an important distinction, as serelaxin's effect on signs and symptoms was predominantly driven by a significant reduction in WHF events. When the analysis of Likert scale is adapted to reflect both improvement and worsening over a 5-day period, the favorable treatment effects of serelaxin on dyspnea relief can be distinguished from placebo (Figure 7-12) (Figure 7-13).

7.3.3.3 Reduction in in-hospital WHF events contributes to positive VAS outcome

In-hospital WHF event was an integral component of the primary VAS endpoint. A comparison of mean VAS scores over time in both treatment groups based on the reported vs. assigned scores showed that the treatment difference in VAS AUC based on actual reported scores (without worst score assignment) was 126.1 mm-hours favoring serelaxin numerically (p=0.3314), compared to a difference of 447.7 mm-hours when calculated using the assigned scores as pre-specified in the study protocol (Figure 7-14). Therefore, the treatment benefit of serelaxin was predominantly driven by a reduction in the occurrence of in-hospital WHF events.

Figure 7-14 Change from baseline of dyspnea VAS - comparison of reported data vs. assigned data from RELAX-AHF (ITT set)



Means							
PBO-reported (N=580)	9.8	14.9	20.6	25.8	27.6	29.6	32.7
RLX-reported (N=581)	10.6	15.7	21.8	26.6	28.5	30.7	33.6
PBO-assigned (N=580)	8.9	13.2	17.1	20.5	21.4	22.4	23.6
RLX-assigned (N=581)	10.2	15.2	20.3	24.2	26.2	26.8	28.2

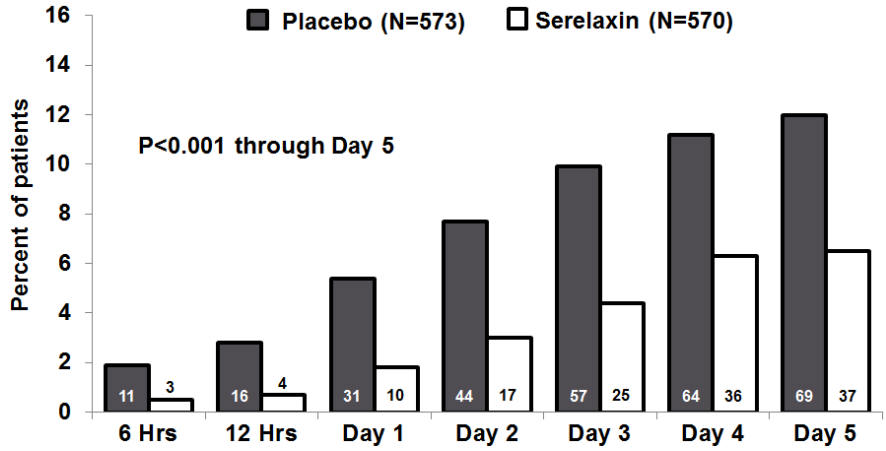
Note: In the placebo-assigned and serelaxin-assigned groups, a patient's reported VAS scores are replaced with the worst post-baseline VAS score from the time of death or a WHF event onward. Missing values are assigned using LOCF and linear interpolation

Serelaxin reduced in-hospital WHF events

A total of 106 patients experienced acute episodes of in-hospital WHF events or died through Day 5, of which most (approximately 95%) were in-hospital WHF events. Significantly fewer patients in the serelaxin group experienced WHF event or death compared to the placebo group (6.5% vs. 12.0% with odds ratio of 0.51; 95% CI: 0.33 to 0.77) (Figure 7-15). The treatment difference was highly statistically significant based on Wilcoxon rank sum test (p=0.00086). Similar results were observed using the time-to-event analysis: the K-M estimate for the risk of experiencing in-hospital WHF or death through Day 5 was significantly lower in the serelaxin group compared with the placebo group (6.7% vs. 12.2%, with HR=0.53, 95% CI: 0.36 to 0.79; p=0.0016 Wald test from Cox regression model) (Figure 7-16).

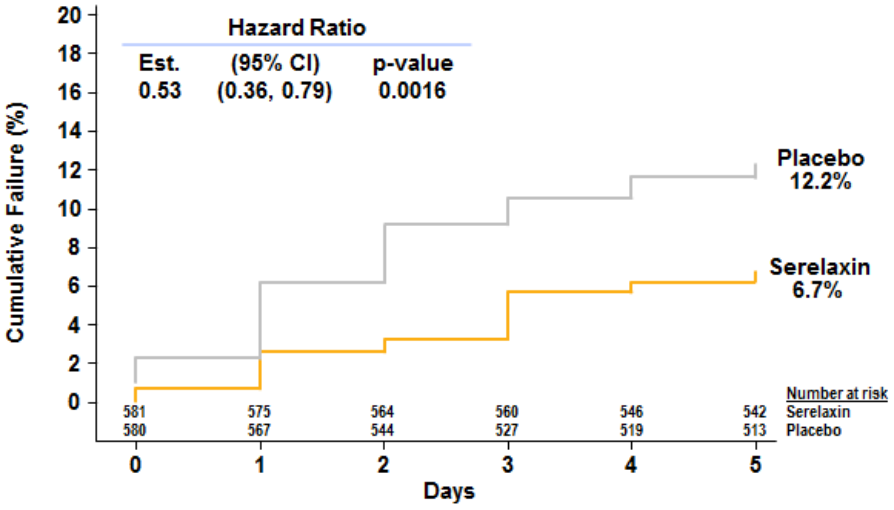
Based on the observed results seen in this study, the number of patients needed to treat (NNT) to prevent one episode of WHF over the period of 5 days is N=19.

Figure 7-15 Cumulative proportion of patients experiencing a worsening heart failure event or death through Day 5 (%) - RELAX-AHF (ITT set)



Numbers of patients with WHF events or death shown for each time point

Figure 7-16 Kaplan-Meier plot of time to worsening heart failure event or death through Day 5 by treatment group - RELAX-AHF (ITT set)



Types of the WHF events characterized by rescue therapies (first event analysis through Day 5) are summarized in Table 7-10. Serelaxin treatment reduced all categories of WHF events, compared to placebo group.

Table 7-10 Number of patients experiencing WHF or death through Day 5 (analysis of first event) – RELAX AHF (ITT set)

	Placebo (N=580)	Serelaxin (N=581)
WHF or death – Total	**69**	**37**
WHF treated with IV loop diuretics only	39*	19
WHF treated with IV nitrates and/or IV diuretics and/or vasodilators only	13	8
WHF treated with inotropes, mechanical ventilation, ultrafiltration or circulatory support	14	7
Death without a prior WHF event	3	3

Analysis is based on first WHF event through Day 5.

*One patient with WHF treated with IV loop diuretics only in the placebo group had a heart failure rehospitalization at Day 4.

Of the 106 patients that developed an in-hospital WHF event through Day 5, 17 patients experienced recurrent events. There were fewer patients that experienced recurrent WHF in the serelaxin group (n=3 for serelaxin vs. n=14 for placebo) (Table 7-11). While the recurrent WHF events that occurred in serelaxin-treated patients were managed with IV loop diuretics, IV nitrates or vasodilators only, the majority of the patients with recurrent events in the placebo group (8/14) required more aggressive treatment with inotropes, mechanical/circulatory support or ultrafiltation (Table 7-11).

Table 7-11 Number of patients experiencing recurrent WHF events by most intensive rescue therapy - RELAX-AHF (ITT set)

	Placebo (N=580)	Serelaxin (N=581)
Number of patients with recurrent WHF	**14**	**3**
IV loop diuretics only	2	2
IV nitrates and/or IV diuretics and/or vasodilators only	4	1
Inotropes, mechanical ventilation, ultrafiltration or circulatory support	8	0

Patients were categorized based on the most intensive therapies for treatment of WHF events

A post-hoc analysis was also performed to compare time to WHF events or death through Day 5 for patients receiving rescue treatment(s) other than IV diuretics alone. If a patient had more than one WHF event, the first event treated with therapy other than IV diuretics was counted in this analysis (Table 7-12). Significantly fewer patients in the serelaxin group (19/570, 3.3%) experienced these events, compared to the placebo group (35/573, 6.1%) (p=0.02487).

Table 7-12 Time to WHF with treatment other than IV diuretics alone through Day 5 (ITT set)

	Statistic	Placebo (N=580)	Serelaxin (N=581)
Cumulative proportion of WHF with treatment other than IV diuretics alone	**n'**	**573**	**570**
6 h	n (%)	6 (1.0)	2 (0.4)
12 h	n (%)	9 (1.6)	3 (0..5)
24 h/Day 1	n (%)	18 (3.1)	7 (1.2)
48 h/Day 2	n (%)	19 (3.3)	8 (1.4)
Day 3	n (%)	28 (4.9)	11 (1.9)
Day 4	n (%)	32 (5.6)	17 (3.0)
Day 5	n (%)	35 (6.1)	19 (3.3)
Time to WHF with treatment other than IV diuretics alone through Day 5 (days)	**n'**	**573**	**570**
	Mean (SD)	5.76 (1.022)	5.89 (0.674)
	95% CI	5.68, 5.84	5.83, 5.94
	Median	6.00	6.00
	Q1, Q3	6.00, 6.00	6.00, 6.00
	Min, Max	0.25, 6.00	0.25, 6.00
	Median difference [1]	0.000	
	95% CI	0.000, 0.000	
	P value [2]	0.02487	

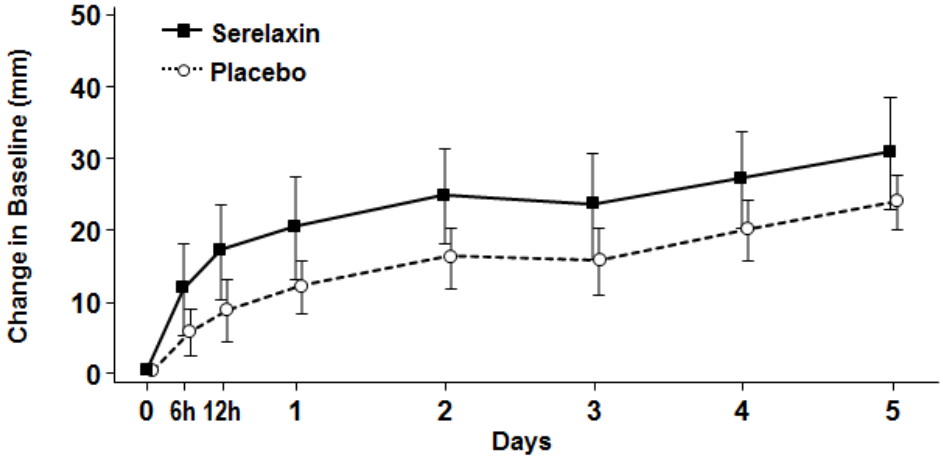
Note: WHF with treatment other than IV diuretics alone includes deaths and WHF events for which patients were not treated with IV diuretics only. Subjects who died by Day 5 without a prior WHF event with treatment other than IV diuretics alone are considered to have an event. Subjects with no events reported through Day 5 are assigned a value of 6 days. If a patient had more than one WHF event, then the first event treated with therapy other than IV diuretics is counted in this analysis.

[1] Hodges-Lehmann estimator of shift

[2] p-value is based on two-sided Wilcoxon rank sum test for serelaxin versus placebo

Finally, in patients that experienced in-hospital WHF events, an analysis of VAS AUC through Day 5 based on the actual reported scores demonstrated that serelaxin treatment was associated with greater VAS AUC, with the mean treatment difference of 829.4 mm-hours vs. placebo (p=0.0806) indicating a greater effect on dyspnea relief in these patients (Figure 7-17).

Figure 7-17 Mean change from baseline (mm) through Day 5 in reported VAS score of WHF patients: RELAX-AHF



Data are presented as mean ± 95% CI

7.3.3.4 In-hospital WHF is an important and distinct clinical event

Patients who experienced a WHF event in RELAX-AHF received persistenly higher doses of IV loop diuretics, had longer length of hospital and ICU/CCU stays and were at increased risk of death.

Characteristics of WHF events

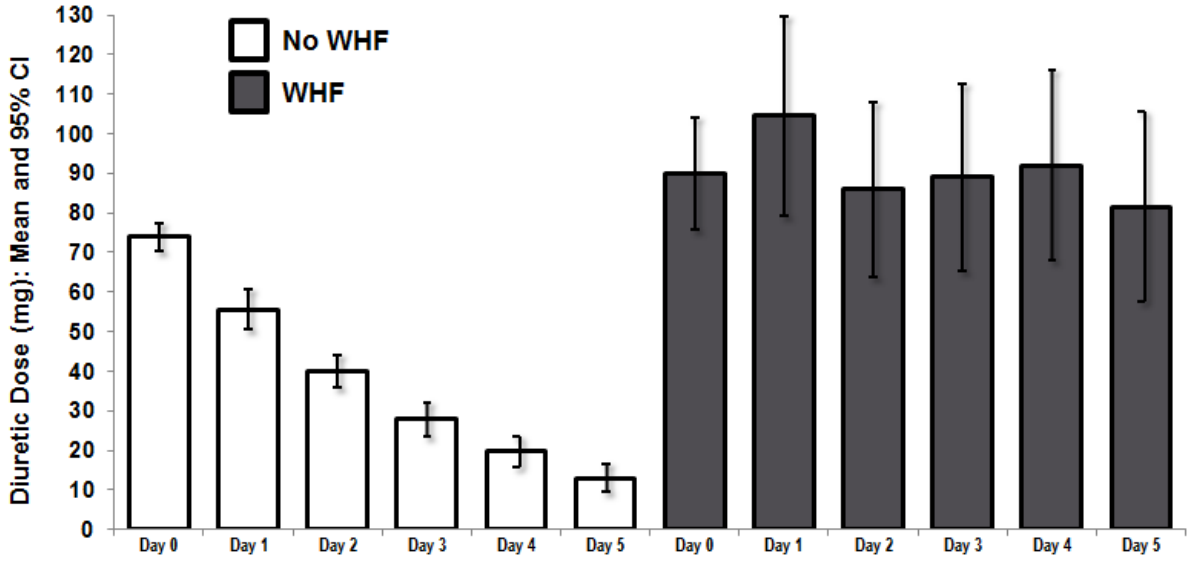
Baseline characteristics are summarized in Table 7-13 for patients who developed in-hospital WHF events or died through Day 5, compared to those without such events. Patients with and without WHF events were clinically similar at baseline.

Table 7-13 Baseline characteristics of patients with or without WHF events or death through Day 5 - RELAX-AHF (ITT set)

Baseline characteristics	WHF (n=106)	Non-WHF (n=1055)
Mean age (years)	72.6	72.0
Male (%)	69	62
Baseline SBP (mmHg)	141	142
Hospitalization for HF in past year (%)	35.8	34.0
CHF 1 month prior to admission (%)	80.2	72.6
LVEF <40% (%)	63.4	53.9
Hypertension (%)	84.9	86.8
Ischemic heart disease (%)	51.9	51.9
Diabetes (%)	50.0	47.2
History of atrial fibrillation or flutter (%)	61.3	50.9
Time – presentation to randomization (hr)	7.6	7.9
VAS score (mm)	44.2	44.2
NT-proBNP (pg/mL)	6146 (n=100)	4963 (n=1002)
hs-Troponin T (µg/L)	0.041 (n=99)	0.034 (n=976)

Following randomization, patients who developed WHF events (irrespective of treatment groups) during the first 5 days required significantly higher doses of daily IV loop diuretics, resulting in significantly greater mean total cumulative IV diuretic doses (Day 1-5) of 447.3 mg compared to 160.9 mg in those without WHF (Figure 7-18).

Figure 7-18 Use of IV loop diuretics (furosemide equivalent dose) in patients with and without WHF through Day 5 - RELAX-AHF (ITT set)



In-hospital WHF events were associated with substantially longer length of initial hospital stay (a difference of 8.0 days) and ICU/CCU stay (a difference of 4.9 days) compared to patients who did not experience such events, which indicates that the occurrence of WHF events fundamentally alters patients' in-hospital clinical course (Table 7-14). In addition, WHF was associated with the worse intermediate- and long-term outcomes. Data from the RELAX-AHF study demonstrated that development of in-hospital WHF events through Day 5 resulted in an HR of 2.27 (p=0.00019) for the risk of CV death or HF/RF hospitalization at Day 60, and an HR of 1.98 (p=0.01479) for the risk of all-cause mortality at Day 180, when compared to patients without WHF (Table 7-15).

Table 7-14 Association between in-hospital WHF event through Day 5 and initial length of hospital stay and ICU/CCU stay - RELAX-AHF (ITT set)

	WHF event through Day 5			
Outcome	**No (N=1055)** Mean (95% CI)	**Yes (N=99)** Mean (95% CI)	**Difference Mean (95% CI)**	**p-value**
Length of initial hospital stay (days)	9.1 (8.6, 9.5)	17.1 (14.1, 20.1)	8.0 (6.3, 9.7)	<0.00001
Length of ICU/CCU stay (days)	3.0 (2.7, 3.3)	7.9 (5.3, 10.4)	4.9 (3.6, 6.1)	<0.00001

Note: Patients who died prior to Day 5 were excluded.

Table 7-15 Relationship between in-hospital WHF event through Day 5 and various clinical outcomes from RELAX-AHF (ITT set)

	WHF event through Day 5			
Outcome	**No (N=1055)*** n (%)	**Yes (N=99)*** n (%)	**HR (95% CI)**	**p-value**
60 days HF/RF readmission or death	122 (11.66%)	25 (25.25%)	2.266 (1.474, 3.484)	0.00019
30 days all-cause mortality	19 (1.81%)	5 (5.05%)	2.858 (1.067, 7.654)	0.03669
60 days CV mortality	30 (2.87%)	9 (9.15%)	3.289 (1.562, 6.928)	0.00173
60 days all-cause mortality	32 (3.06%)	10 (10.10%)	3.424 (1.683, 6.966)	0.00068
180 days CV mortality	69 (6.64%)	12 (12.25%)	1.946 (1.054, 3.592)	0.03328
180 days all-cause mortality	85 (8.15%)	15 (15.18%)	1.979 (1.143, 3.427)	0.01479

Note: Patients who died prior to Day 5 were excluded.

* Presented as number of patients with event (KM estimate of event rate).

For the subset of patients who developed WHF events and were treated only with IV diuretics (N=39 in placebo vs. N=19 in serelaxin), there was also an important and clinically relevant increase in initial LoS in hospital and ICU/CCU stay by 5.4 days and 2.9 days, respectively, when compared with patients who did not experience an in-hospital WHF event (Table 7-16). Moreover, higher risks for Day 60 CV death or HF/RF hospitalization (HR: 1.98, p=0.0190) and Day 180 all-cause mortality (HR: 1.53, p=0.2822) were reported for this subset of WHF patients.

Table 7-16 Association between in-hospital WHF event treated with IV diuretics only through Day 5 and initial length of hospital stay and ICU/CCU stay - RELAX-AHF (ITT set)

	WHF event treated with IV diuretics only through Day 5			
Outcome	**No (N=1055)** Mean (95% CI)	**Yes (N=58)** Mean (95% CI)	**Difference** Mean (95% CI)	**p-value**
Length of initial hospital stay (days)	9.1 (8.6, 9.5)	14.5 (11.0, 18.0)	5.4 (3.4, 7.5)	<0.00001
Length of ICU/CCU stay (days)	3.0 (2.7, 3.3)	5.9 (3.2, 8.7)	2.9 (1.5, 4.3)	0.00005

Note: Excludes patients who died prior to Day 5

In conclusion, these results suggest that patients who developed in-hospital WHF events, including those who were treated only with IV diuretics, were characterized by deterioration of their in-hospital clinical course, as reflected by a significant increase in the LoS for index hospitalization and length of ICU/CCU stay. Patients who experienced WHF events were also associated with higher risk of intermediate- and longer-term HF morbidity and mortality.

7.3.3.5 Supportive sensitivity analyses on VAS AUC

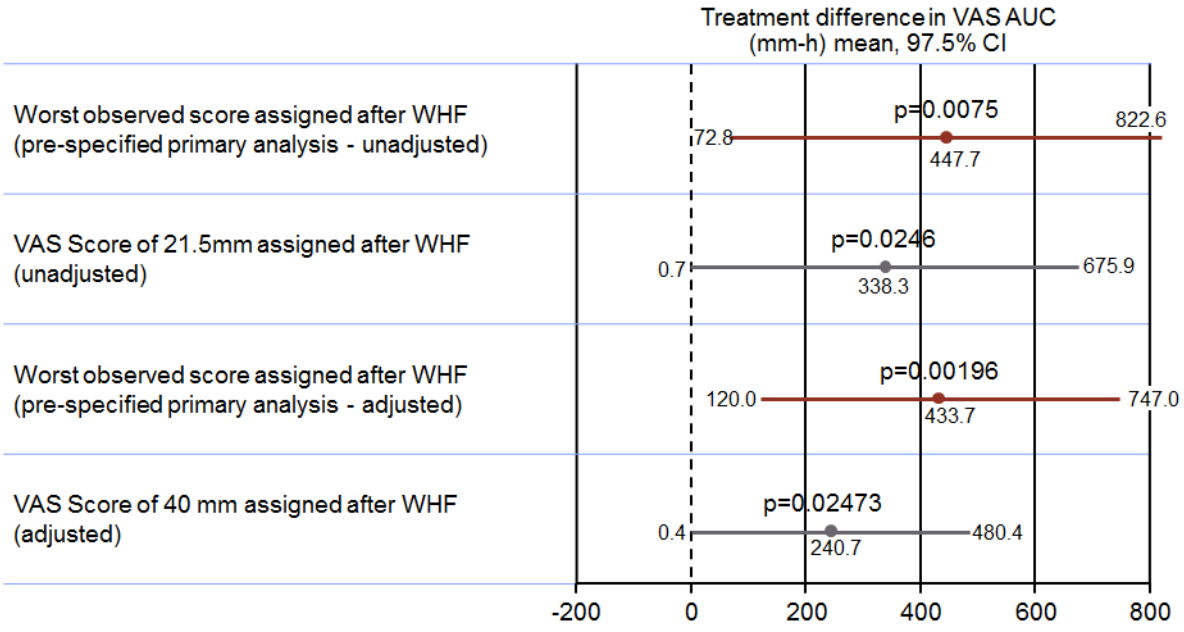
Tipping point analyses of assigned VAS score post-WHF

Several pre-specified and post-hoc sensitivity analyses were performed on the primary endpoint of VAS AUC Day 0-5, assigning scores other than the pre-specified worst-reported score (VAS = 0) to those in-hospital WHF events.

The first sensitivity analysis was pre-specified in the RELAX-AHF study protocol which assigned the baseline VAS scores reported from the individual WHF patients to all the timepoints after event onset. This analysis showed a non-significant treatment effect of 174.4 mm-hours favoring serelaxin (95% CI: -122.8 to 471.6 mm-hours, p=0.1828 vs. placebo). It should be noted that the baseline VAS score reflects the initial improvement that took place from the time of initial presentation to randomization. Therefore, it does not reflect the severity of the presenting or recurrent symptoms.

A post-hoc tipping point analysis was performed to explore the maximum VAS score assigned to all measurements after a patient developed an in-hospital WHF event that would still maintain a p-value below the threshold of statistical significance. The results showed that, by assigning any value of up to 21.5 mm for all VAS scores following WHF episodes, the p-value remained below 0.025 (Figure 7-19). This value of 21.5 mm corresponds to approximately half of the mean reported baseline VAS score of 44.2 mm. Moreover, a covariate-adjusted analysis using baseline VAS decile in addition to age, race, gender and country demonstrates that an assigned score of up to 40 mm, which is only slightly lower than the mean reported baseline score, would still preserve the statistical significance (Figure 7-19).

Figure 7-19 Tipping point analysis of assigned VAS score post-WHF



Note: Rule of assigning worst score after death was retained in all sensitivity analyses

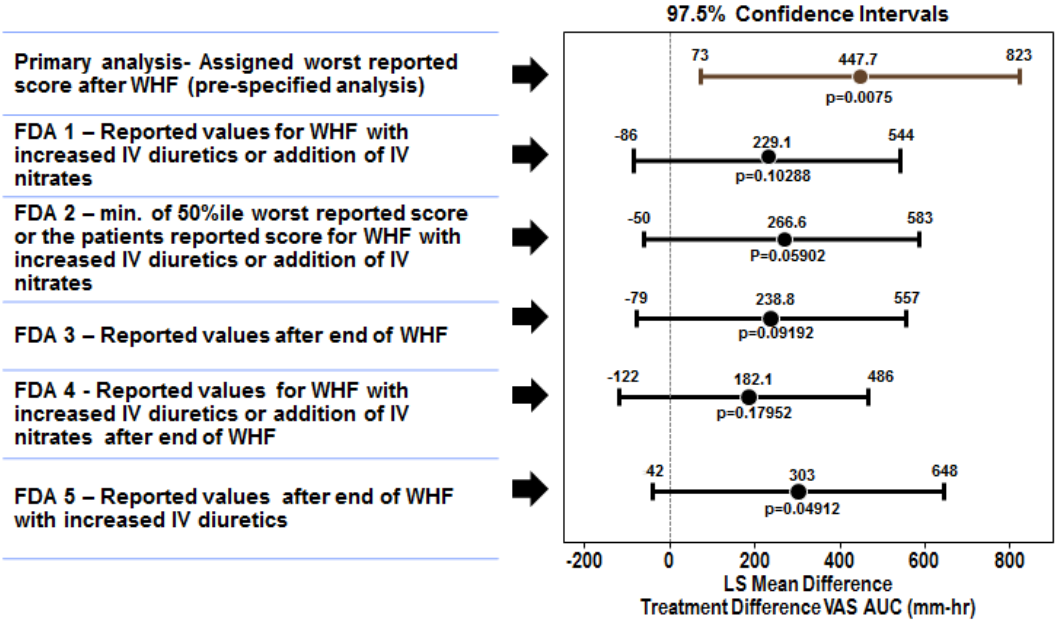
Adjusted analysis based on ANCOVA with treatment group, country, age, gender, race and baseline dyspnea VAS

Tipping point analysis for WHF events treated with IV diuretics only: A tipping point analysis that varied the assigned VAS scores for WHF patients who were treated with IV diuretics only (n=58) was also performed. The worst reported score (VAS = 0) was still assigned for all WHF patients who died or received other rescue therapies. This analysis demonstrated that assigning a score up to 34 mm or 56 mm for a WHF event treated with IV loop diuretics only would still maintain the statistical significance based on the unadjusted or covariate-adjusted analyses, respectively.

FDA-requested VAS sensitivity analyses

At the request of the Agency, several additional sensitivity analyses were performed to assess the robustness of the RELAX-AHF finding with respect to different ways of weighting the importance of WHF including taking the actual duration of in-hospital WHF events into account and their impact on the VAS AUC change from baseline through Day 5 (Figure 7-20). The point estimates resulting from these sensitivity analyses consistently favor serelaxin, irrespective of the approaches used to assign scores after the occurrence of WHF events.

Figure 7-20 FDA-requested VAS sensitivity analyses – unadjusted analyses



Note: Rule of assigning the worst reported score after death was retained in all sensitivity analyses

FDA 1: Dyspnea score assignment stated in the protocol [worst reported value for any patient with WHF, i.e., 0, carried forward] except for patients who had WHF with increased IV diuretics or addition of IV nitrates only, for whom reported values were used.

FDA 2: Dyspnea score assignment stated in the protocol except for patients who had WHF with increased IV diuretics or addition of IV nitrates only, for whom the minimum of the median reported value and the patient's reported value was used.

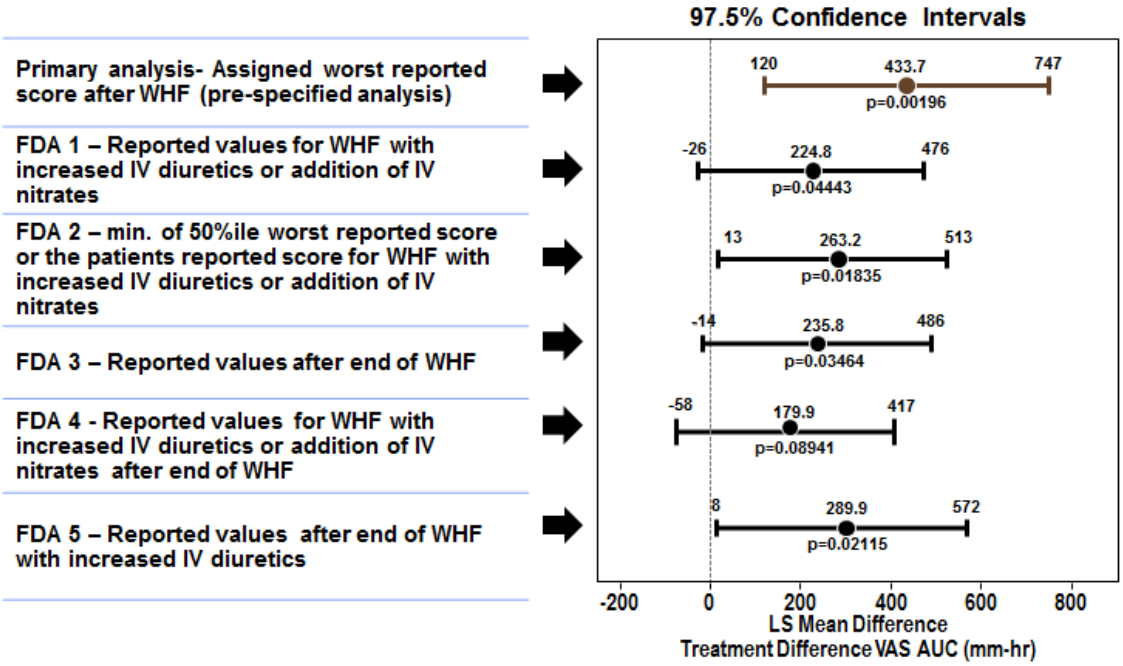
FDA 3: Assign a 0 for any VAS assessment time point where there was an actual WHF event but did not carry it forward, unless that WHF event was continuing, i.e., for all VAS assessment time points subsequent to the completion of a WHF event, the reported scores were used.

FDA 4: Assign a 0 for any VAS assessment time point where there was an actual WHF event except for cases with increased IV diuretics or addition of IV nitrates only (when reported VAS scores were used) but did not carry it forward, unless that WHF event was continuing, i.e., for all VAS assessment time points subsequent to the completion of a WHF event, the reported score was used.

FDA 5: Assign a 0 value during the duration of the event of the WHF episode for patients who received IV diuretics (no treatment or inhaler; but not pressors, IV nitrates, milrinone, ultrafiltration, rehospitalization, intubation and death) and reported scores after end of WHF episode. For all other patients use worst score assignment.

In addition, the covariate-adjusted sensitivity analyses were performed which were pre-specified in the statistical analysis plan (Figure 7-21). The results of these covariate-adjusted analyses were applied to each one of sensitivity analyses summarized in Figure 7-20. Using baseline VAS decile in addition to age, gender, race, and country as covariates, the results of these covariate-adjusted analyses were consistent with the pre-specified primary analysis.

Figure 7-21 FDA-requested VAS sensitivity analyses – adjusted for covariates (including baseline VAS decile)



Note: Rule of assigning the worst reported score after death was retained in all sensitivity analyses. Based on ANCOVA with treatment group, country, age, gender, race and baseline dyspnea VAS decile as covariates.

VAS sensitivity analyses based on non-parametric tests

Further post-hoc sensitivity analyses of the dyspnea VAS endpoint based on non-parametric log-rank tests were conducted on modified rankings for VAS AUC where the highest ranks were assigned to the worst outcome. The log-rank test is based on ranks like the Wilcoxon rank-sum tests, but is more sensitive to treatment differences on the more unfavorable side of the distribution. The Wilcoxon rank-sum test is sensitive to detect a shift in the median but down-weights the higher ranks, i.e., severe deterioration such as those resulting from WHF events (or death). In order to retain a more appropriate weighting of WHF events and death, while providing an assessment that does not depend on a particular value assigned after a WHF event, the following rank analyses for VAS AUC were assessed:

- Log-rank test applied to AUC of VAS change from baseline (worst AUCs receive highest ranks)

- Log-rank test applied to a modified ranking of AUC of VAS change from baseline (following the ideas of Finkelstein and Schoenfeld, 1999: deaths are ranked first (earlier deaths have highest ranks) and WHF events are ranked next (earlier WHF events have highest ranks). Finally, patients without WHF events/death are ranked below; worse outcomes (large negative AUCs) are assigned highest ranks.

Multivariate analysis (following ideas of Saville et al 2010 and Wei et al 1989): define ranked score for each assessment time point. Deaths have the highest ranks, WHF events have the second highest ranks, and patients without WHF events/death are ranked below, such that lower VAS scores have higher ranks. The log hazard ratio (HR) is estimated for each time point and then the weighted average of the estimated log HR with weights as in the AUC calculation (0.25/5 for 6 hours and 12 hours, 0.5/5 for 24 hours, 1/5 for Days 2, 3, 4 and 5) is calculated along with its estimated variance, and then the corresponding p-value is determined based on the Wald test.These non-parametric sensitivity analyses show similar results as the pre-specified t-test used for the primary analysis (see Table 7-17).

Table 7-17 Primary dyspnea VAS endpoint (sensitivity analyses based on non-parametric tests are consistent with primary analysis results)

AUC of VAS change from baseline through Day 5	
	p-value
Primary efficacy analysis based on t-test	0.0075
Wilcoxon rank-sum test	0.0819
Randomization-based t-test	0.0075
Log-rank test applied to AUC of VAS change from baseline	0.007
Log-rank test applied to a modified ranking of AUC of VAS change from baseline (following ideas of Finkelstein & Schoenfeld, 1999)	0.011
Multivariate analysis (following Saville et al 2010 and Wei et al 1989)	
- Unadjusted	0.0014
- Adjusted for baseline VAS	0.0001

A further analysis of AUC of VAS change from baseline with the distribution-free randomization-based t-test (as originally planned in the protocol) with 100,000 random permutations resulted in a p=0.00749 (reported as p=0.0075 in Table 7-17), virtually identical to the primary analysis results based on t-distribution (p=0.00746) (reported as p=0.0075 in Table 7-17), demonstrating the robustness of primary analysis results against deparures from normality.

7.3.3.6 Conclusion: serelaxin achieved dyspnea relief assessed by VAS through reduction in WHF

The analyses evaluating the primary efficacy endpoint of VAS AUC through Day 5 indicate that prevention of in-hospital WHF events primarily contributed to the observed benefit on dyspnea relief. The reduction of WHF is important because the WHF event represents clinically important symptom deterioration and is associated with significantly longer in-hospital LoS (mean difference of 8 days vs. non-WHF) and also ICU/CCU stay (mean difference of 4.9 days vs. non-WHF), indicating that the occurrence of WHF episode meaningfully alters the patient's in-hospital clinical course. In addition, consistent with findings from several other AHF trials, in-hospital WHF events through Day 5 were associated with an increased risk of mortality in the RELAX-AHF study.

Several pre-specified and post-hoc sensitivity analyses were performed on the primary VAS AUC endpoint using various approaches to analyze occurrence of WHF, including assigning scores other than the pre-specified worst score (VAS = 0) to WHF events. Overall, the results of sensitivity analyses were consistent with the pre-specified primary analysis.

7.3.4 Secondary efficacy outcomes

7.3.4.1 Cardiovascular death or re-hospitalization due to heart failure or renal failure through Day 60

The composite of CV death or re-hospitalization due to HF or RF through Day 60 was one of two pre-specified secondary efficacy endpoints in RELAX-AHF study, but the study was not powered for this specific endpoint. The time to the first event of CV death or re-hospitalization due to HF or RF through Day 60 in the ITT set did not differ significantly between the serelaxin and placebo groups (HR=1.02, 95% CI: 0.74 to 1.41, p=0.8945) (Table 7-18). Analysis of the individual component 'CV death' showed 27 events for placebo vs. 19 events for serelaxin, while conversely the component of re-hospitalization due to HF or RF showed 50 events for placebo vs. 60 events for serelaxin. Neither of these differences was statistically significant.

Table 7-18 Cardiovascular death or re-hospitalization due to heart failure or renal failure through Day 60 - RELAX-AHF (ITT set)

	Statistic	Placebo N=580	Serelaxin N=581
Number of events	n (%)	75 (12.9)	76 (13.1)
K-M estimates	Probability (95% CI)		
	Day 5	1.0 (0.5, 2.3)	0.7 (0.3, 1.8)
	Day 14	3.5 (2.2, 5.3)	3.3 (2.1, 5.1)
	Day 30	6.9 (5.1, 9.3)	7.5 ((5.6, 9.9)
	Day 60	13.0 (10.5, 16.1)	13.2 (10.7, 16.3)
	p-value		0.8945
Estimates by Cox model	Hazard Ratio (95% CI) serelaxin vs. placebo		1.02 (0.74, 1.41)
Composite event components			
CV death	n (%)	27 (4.7)	19 (3.3)
	Probability (95% CI) Day 60	4.7 (3.2, 6.8)	3.3 (2.1, 5.1)
	p-value		0.2278
	Hazard Ratio (95% CI) serelaxin vs. placebo		0.70 (0.39, 1.26)
HF/RF rehospitalization	n (%)	50 (8.6)	60 (10.3)
	Probability (95% CI) Day 60	9.0 (6.9, 11.7)	10.6 (8.3, 13.5)
	p-value		0.3166
	Hazard Ratio (95% CI) serelaxin vs. placebo		1.21 (0.83, 1.76)

The observed numerical differences in re-hospitalizations may be explained by the concept of competing risk: the larger number of patient deaths in the placebo group removed sicker patients (those who were at higher risk for hospitalization) from the study in this group before they could be re-hospitalized. This hypothesis is supported by an additional analysis of composite of all-cause death or re-hospitalization due to HF or RF which can be interpreted as a re-hospitalization analysis that is adjusted for competing risk of death. The proportion of all-cause death or re-hospitalization due to HF or RF through Day 60 was identical in both treatment groups (Kaplan-Meier estimates: serelaxin 13.4%, placebo 13.4%; HR=1.01).

7.3.4.2 Days alive and out of hospital through Day 60

The composite endpoint of DAOOH was the other secondary efficacy endpoint in RELAX-AHF. DAOOH through Day 60 was computed as 61 days minus the days in hospital through Day 60 (including the index hospitalization and any re-hospitalizations), and minus the days following a death prior to Day 60. The mean number of DAOOH was similar in the serelaxin group compared to the placebo group (48.3 vs. 47.7 days, p=0.3682) (Table 7-19).

Table 7-19 Days alive and out of hospital through Day 60 - RELAX-AHF (ITT set)

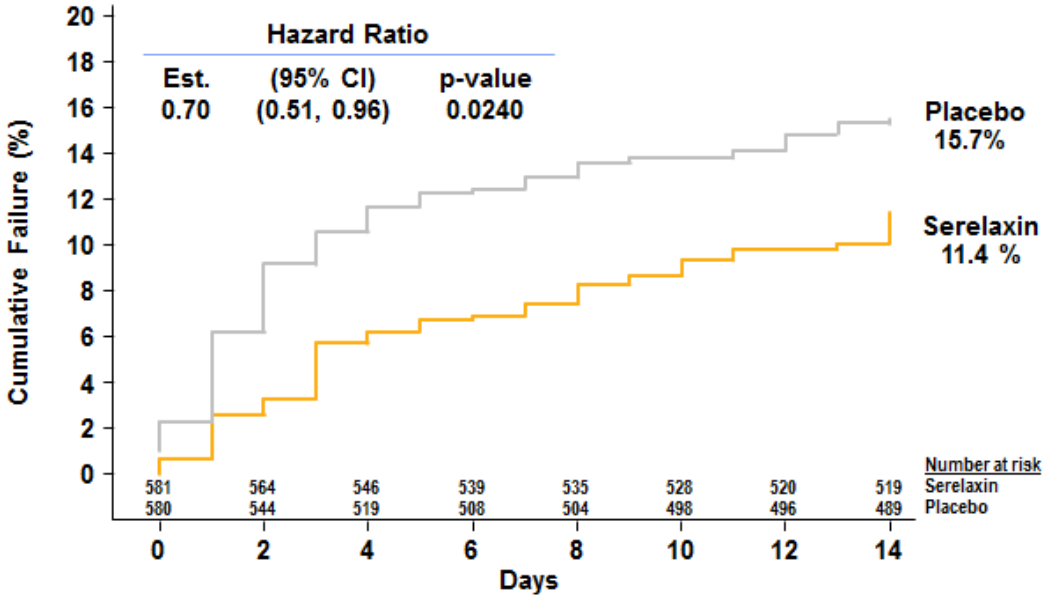
Statistic	Placebo N=580	Serelaxin N=581
Mean (SD)	47.7 (12.11)	48.3 (11.59)
95% CI	46.7, 48.7	47.3, 49.2
Median	52	52
Q1, Q3	45.0, 55.0	46.0, 55.0
p-value *		0.3682

* P-value is based on two-sided Wilcoxon rank sum test for serelaxin vs. placebo

7.3.5 Worsening heart failure through Day 14

The combined risk of death, rehospitalization for HF and in-hospital WHF through Day 14 was a pre-specified efficacy endpoint. Serelaxin was associated with significantly reduced risk of this composite endpoint through Day 14 (11.4% vs. 15.7%) with HR=0.70 (95% CI 0.51 to 0.96, p=0.024) (Figure 7-22).

Figure 7-22 Kaplan-Meier plot of time to the composite worsening heart failure endpoint through Day 14 – RELAX-AHF (ITT set)

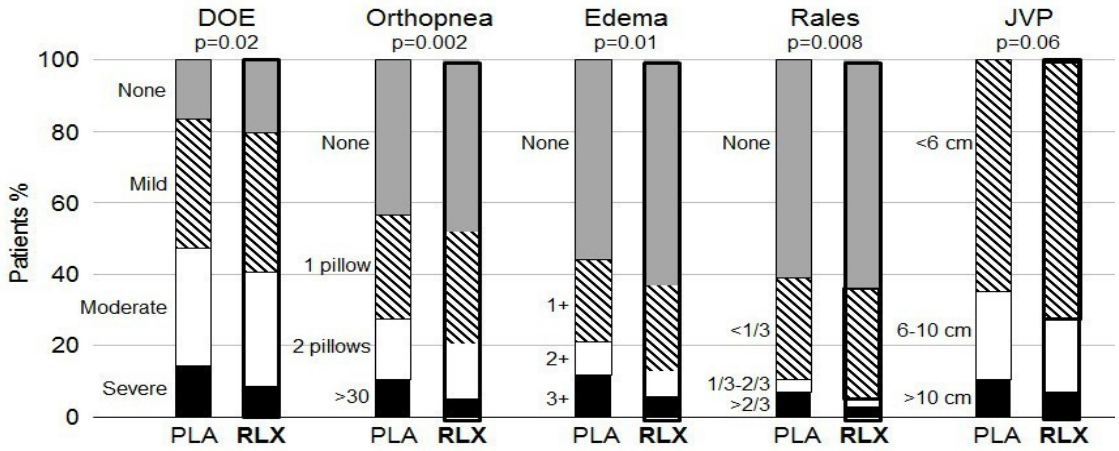


7.3.6 Physician assessment of HF signs and symptoms

All patients randomized to the RELAX-AHF study had signs and symptoms of congestion prior to study entry, and the distributions for the severities of pre-specified physician-assessed signs and symptoms of congestion (i.e., dyspnea on exertion, orthopnea, edema and rales on a 4-point scale, and JVP on a 3-point scale) were similar in both the serelaxin and placebo groups at baseline.

An analysis was performed to evaluate the treatment differences in the distributions for the severities of signs and symptoms of congestion. A pre-specified worst score assignment was also applied for these analyses in an event of in-hospital WHF or death. Serelaxin treatment was associated with significant improvement in most of the signs and symptoms of congestion at Days 2 (Figure 7-23) and 5, compared to placebo.

Figure 7-23 Physician-assessed signs and symptoms of congestion at Day 2 – RELAX-AHF (ITT set)



p value by 2-sided Wilcoxon rank sum test for RLX vs. PLA

A time-to-first event analysis using a Cox model (response defined as ≥1 point improvement from baseline) was applied to provide an effect size for the treatment comparisons in physician-assessed HF signs and symptoms through Day 5 (Table 7-20). Effect sizes were in the range of 19-33% relative improvement in serelaxin patients, except for jugular venous pressure.

Table 7-20 Treatment comparisons of physician-assessed HF signs and symptoms through Day 5 - RELAX-AHF (ITT set)

HF signs/symptoms	Models	Estimate[1] (95% CI)	p-value[1]
Dyspnea on exertion	Time-to-event[1]	1.224 (1.050, 1.428)	0.0095
Orthopnea	Time-to-event[1]	1.232 (1.052, 1.443)	0.0095
Edema	Time-to-event[1]	1.331 (1.124, 1.577)	0.0011
Rales	Time-to-event[1]	1.189 (1.013, 1.396)	0.0286
Jugular venous pressure	Time-to-event[1]	1.098 (0.924, 1.304)	0.2786

1 - Cox model using treatment as factor and stratified by regions; estimates are hazard ratios (serelaxin/placebo) with values >1 indicating effect in favor of serelaxin; p values are from log rank test

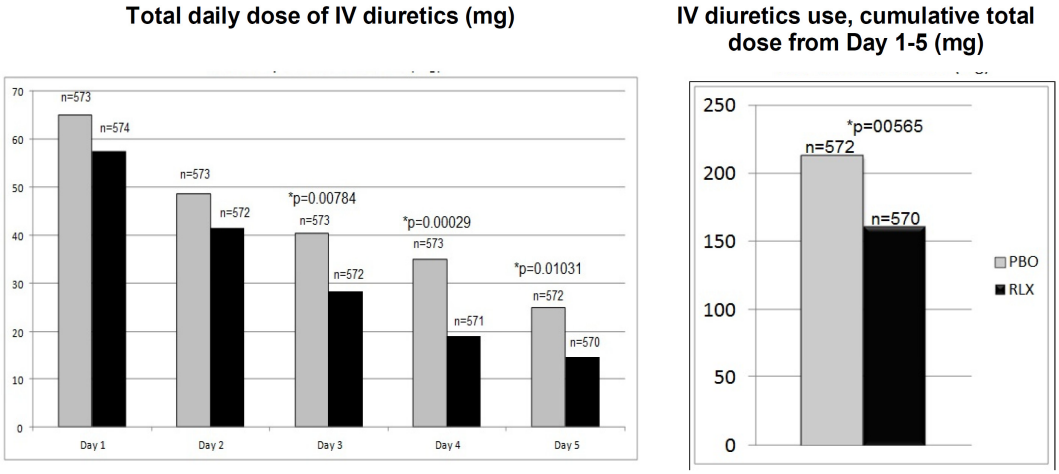
7.3.7 Use of IV loop diuretics and other IV HF therapies

7.3.7.1 Daily and total dose of IV loop diuretics through Day 5

Once a patient was randomized in RELAX-AHF study, physicians were free to administer IV loop diuretics and other IV therapies according to their clinical judgment. The total doses of IV loop diuretics through Day 5 or discharge, whichever was earlier, were assessed as a pre-specified efficacy endpoint to evaluate whether the effects of serelaxin could be accounted for by greater use of concomitant therapy for treatment of AHF.

The daily and cumulative total doses of IV diuretics through Day 5 by treatment group are shown in Figure 7-24. Per protocol, all patients had to have received ≥40 mg IV furosemide (or equivalent) prior to randomization, and the doses shown in Figure 7-24 were given in addition to this dose. Compared to placebo, the serelaxin group had numerically lower IV diuretic use on all hospital days, and this was statistically significant on Days 3, 4 and 5 ($p<0.05$, t-test). There was also a statistically significantly lower mean total cumulative dose of IV diuretics used from Day 1 through Day 5 (placebo: 213.0 mg vs. serelaxin: 161.3 mg, p=0.0057).

Figure 7-24 Total daily dose of IV diuretics (mg) from Day 1 to Day 5 - RELAX-AHF (ITT set)



* p-values by t-test are shown for time points where p < 0.05. P values not shown if > 0.05.

Calculation of furosemide equivalents (mg) for torsemide, bumetanide and ethacrynic acid are actual dose (mg) multiplied by constant 2, 20 or 0.8, respectively; each time point reflects medication use within prior 24 hrs.

Further analysis demonstrated that the difference in the cumulative dose of IV diuretics during the first 5 days was mainly driven by less diuretic use among the serelaxin patients in patients without WHF events (182.5 mg in placebo vs. 140.4 mg in serelaxin; mean difference: -42.1 mg, p=0.0151).

7.3.7.2 Time to permanent discontinuation of IV diuretics through Day 5

An important factor in hospital discharge planning is the discontinuation of IV diuretics and transition to oral diuretics. A more rapid discontinuation of IV diuretics in the hospital setting would be consistent with clinical improvement and would be expected to have a favorable impact on the length of hospital stay. Additional analyses using Kaplan-Meier (K-M) estimates of the time to first day of permanent discontinuation of IV diuretics from Day 1 through Day 5 (Table 7-21) demonstrated that patients on serelaxin were more likely to permanently discontinue IV diuretics at an earlier point during their hospitalization than patients on placebo (HR=1.24 favoring serelaxin, p=0.0001).

Table 7-21 Time to first day of permanent discontinuation of IV loop diuretics through Day 5 - RELAX-AHF (ITT set)

	Statistic		Placebo (N=580)	Serelaxin (N=581)
No. events	n (%)		446 (76.9)	495 (85.2)
% patients off IV loop diuretics (Kaplan Meier estimate)	Probability (95% CI)	Day 1	24.3 (21.0, 28.0)	26.8 (23.4, 30.7)
		Day 2	39.6 (35.7, 43.7)	46.2 (42.2, 50.4)
		Day 3	56.7 (52.7, 60.8)	64.7 (60.8, 68.6)
		Day 4	67.7 (63.9, 71.5)	78.5 (75.1, 81.8)
		Day 5	77.9 (74.4, 81.2)	86.4 (83.4, 89.0)
	p-value			0.0001
Estimates (Cox model)	HR (95% CI) serelaxin vs placebo			1.24 (1.09, 1.40)

P-value is based on log-rank test for serelaxin vs placebo. HR >1.0 favors serelaxin.

Patients are only counted as having an event if they remained off IV loop diuretics through Day 5. Patients who died prior to Day 5 assessment are censored at Day 5. Patients who withdrew consent while on IV loop diuretics are censored at day of withdrawal.

7.3.7.3 Other IV HF therapies

Fewer serelaxin-treated patients used IV nitroglycerin (12.4% placebo vs. 9.5% serelaxin) and/or IV inotropic agents including dopamine (2.6% placebo vs. 1.4% serelaxin) or dobutamine (2.4% placebo vs. 0.9% serelaxin) from Day 1 through Day 5.

7.3.8 Length of hospital and CCU/ICU stay during index hospitalization

The length of stay (LOS) for both the index hospitalization and ICU/CCU were assessed as a pre-specified efficacy endpoints in the Phase III RELAX-AHF study. The LOS during index hospitalization was significantly reduced for serelaxin compared to placebo (p=0.039 based on a Wilcoxon rank sum test). The mean length of hospital stay was shorter in the serelaxin treatment group (9.6 days) compared to the placebo group (10.5 days), with a mean treatment difference of 0.9 days. In addition, a reduction in duration of ICU/CCU stay was also observed in the serelaxin group (p=0.029 based on a Wilcoxon rank sum test) with mean durations of 3.5 days in the serelaxin group vs. 3.9 days in the placebo group.

7.3.9 Changes in CV and renal biomarkers through Day 14

In RELAX-AHF, measurements of hs-cTnT, cystatin-C and NT-proBNP were performed to evaluate the degree of myocardial injury, renal impairment and ventricular wall stress, respectively, in AHF patients. These biomarkers were measured at baseline, Day 2, Day 5, and Day 14 by an independent central laboratory. The definitions of worsening in end-organ functions were determined post-hoc based on current literature recommendations as following:

- Myocardial necrosis was defined as an increase in hs-cTnT levels from baseline to Day 2 by ≥20% (Thygesen et al 2012b, Januzzi et al 2012).
- Meaningful decrease in cardiac wall stress was defined as decrease in NT-proBNP from baseline to Day 2 by ≥30% (Bettencourt et al 2004, Maisel et al 2008).

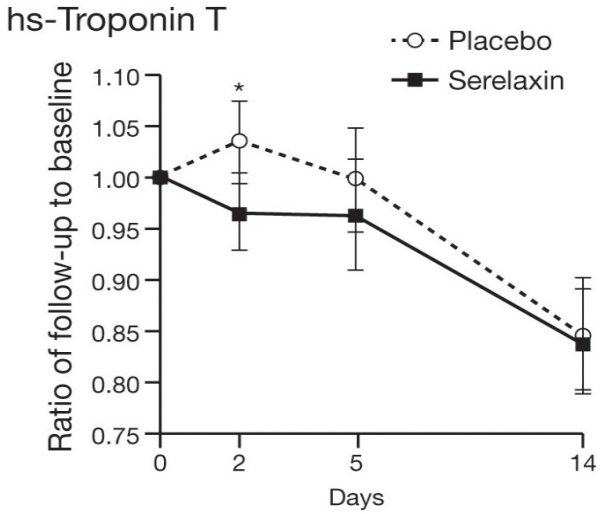
- Worsening renal function (WRF) was defined as an increase from baseline to Day 2 in plasma cystatin-C values by ≥0.3 mg/L (22 nmol/L), or in serum creatinine by > 0.3 mg/dL (27 µmol/L), (Metra et al 2013, Gottlieb et al 2002, Lassus et al 2007).

7.3.9.1 Myocardial injury

In RELAX-AHF study, the baseline hs-cTnT plasma levels were above the 99th percentile of upper reference limit in 90% of patients, and an increase ≥ 20% over baseline at Day 2 was associated with a HR (95% CI) of 1.80 (1.16-2.78) in 180-day mortality (p=0.0076).

As shown in Figure 7-25, serelaxin treatment, compared to placebo, was associated with a significantly smaller change from baseline in hs-cTnT levels at Day 2 (p = 0.013). In addition, fewer patients had a substantial further increase (≥ 20% above baseline) in hs-cTnT levels at Day 2 (16.5% vs. 27.2%, p<0.0001). These observations suggest that 48-hour infusion of serelaxin may protect against myocardial injury early in the clinical course of AHF.

Figure 7-25 Changes from baseline through Days 2-14 in hs-troponin-T – RELAX-AHF



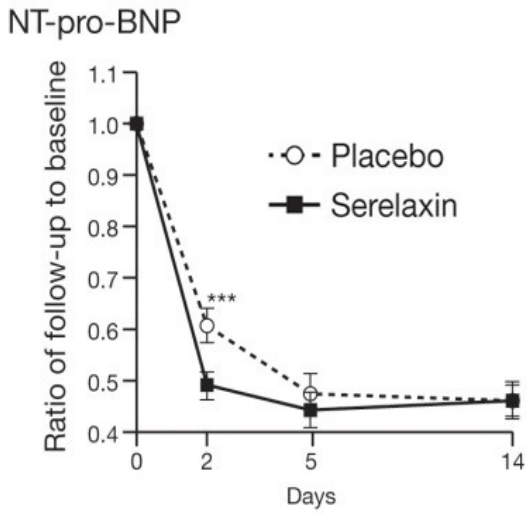
* p<0.05 by repeated – measures analysis of variance with adjustment for baseline value

7.3.9.2 Cardiac wall stress

In RELAX-AHF, higher NT-proBNP levels were associated with increased 180-day CV mortality risk; on the other hand, the reduction during the first 2 days from hospitalization predicted better prognosis, e.g., a decrease in NT-proBNP ≥30% from baseline to Day 2 was associated with a HR (95% CI) of 0.47 (0.31-0.69) in 180-day mortality (p=0.0001).

Serelaxin treatment resulted in significantly lower NT-proBNP levels at Day 2 (p <0.0001) (Figure 7-26). It was also associated with a greater proportion of patients having a ≥30% decrease in NT-proBNP from baseline to Day 2, compared to placebo (placebo 58.0% vs. serelaxin 69.0%, p = 0.0002).

Figure 7-26 Changes from baseline through Days 2-14 in NT-proBNP - RELAX-AHF



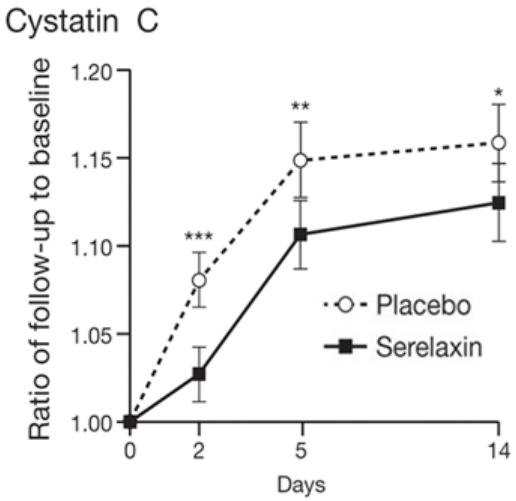
p<0.001 by repeated – measures analysis of variance with adjustment for baseline value

7.3.9.3 Worsening renal function

In RELAX-AHF study, WRF defined as cystatin-C increase from baseline by ≥0.3 mg/dL (22 nmol/L) or serum creatinine increase by ≥0.3 mg/dL (27 µmol/L), occurred in 15.4% and 19.6% of patients at Day 2, respectively. An increase in cystatin-C ≥0.3 mg/dL (22 nmol/L) over baseline at Day 2 was associated with a HR (95% CI) of 2.10 (1.38-3.20) in 180-day mortality (p=0.0004).

Significantly lower plasma cystatin-C values were observed at as earlier as Day 2 in serelaxin patients, and it remained persistently lower than the placebo patients through Days 5 and 14 (Figure 7-27). A similar reduction in serum creatinine was also observed through Day 5. In addition, serelaxin administration was associated with a lower incidence of WRF at Day 2 with a HR (95% CI) of 0.63 (0.46-0.85) by cystatin-C criterion (p=0.0027), and a HR (95% CI) of 0.50 (0.35-0.70) by creatinine criterion (p<0.0001) (Metra et al 2013). The significantly lower levels of plasma cystatin-C in the serelaxin group were also maintained at Day 60. This finding demonstrated that serelaxin treatment prevented early renal dysfunction.

Figure 7-27 Changes from baseline through Days 2-14 in cystatin-C



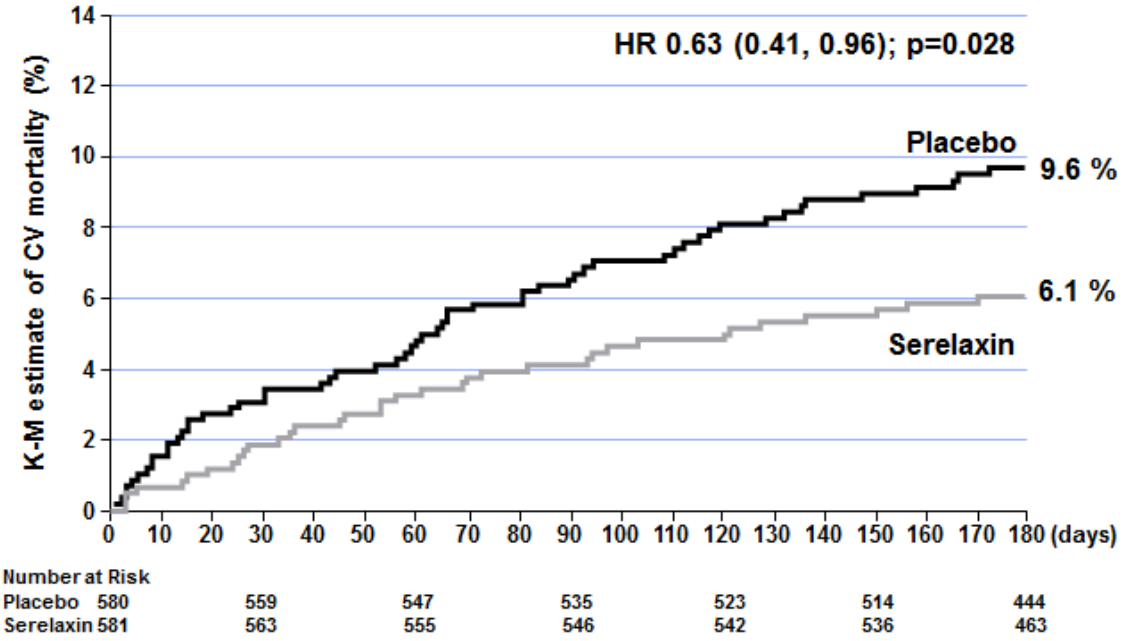
* p=0.0159 by repeated – measures analysis of variance with adjustment for baseline value
** p=0.0028
*** p<0.0001

7.3.10 Cardiovascular mortality

The 180-day CV mortality was a pre-specified efficacy endpoint in the RELAX-AHF study. The proportion of patients with CV death through Day 180 was statistically significantly lower in the serelaxin group compared to the placebo group (K-M estimate 6.1% vs. 9.6%) (Figure 7-28) (Table 7-22). The hazard ratio using a Cox model showed that patients in the serelaxin group had a lower risk of CV death through Day 180 with a HR (95% CI) of 0.63 (0.41-0.96), i.e., a 37% relative risk reduction (p=0.028). The K-M curves for CV death began to diverge early, with a lower rate of CV death in serelaxin group evident from as early as Day 5 onwards, compared to placebo.

In the RELAX-AHF protocol, causes of deaths through Day 60 were independently adjudicated while causes of deaths occurring after Day 60 were assessed by the investigator. A post-hoc adjudication of those deaths that occurred between Day 61 and Day 180 was performed by the same CEC while still blinded to the study results. The overall rate of adjudicated CV death through Day 180 (K-M estimate 5.9% in serelaxin vs. 9.4% in placebo) was similar to the rate determined following the protocol pre-specified algorithm.

Figure 7-28 Cardiovascular mortaility through Day 180 - RELAX-AHF (ITT set)



* Adjudicated up to Day 60

Table 7-22 Cardiovascular death through Day 180 - RELAX-AHF (ITT set)

	Statistic	Placebo N=580	Serelaxin N=581
Number of events	n (%)	55 (9.5)	35 (6.0)
Kaplan-Meier estimates for time to cardiovascular death (days)	Probability (95% CI)		
	Day 5	0.9 (0.4, 2.1)	0.7 (0.3, 1.8)
	Day 14	2.1 (1.2, 3.6)	0.9 (0.4, 2.1)
	Day 30	3.3 (2.1, 5.1)	1.9 (1.1, 3.4)
	Day 60	4.7 (3.2, 6.8)	3.3 (2.1, 5.1)
	Day 180	9.6 (7.5, 12.3)	6.1 (4.4, 8.4)
	P-value		0.028
Estimates by Cox model	Hazard ratio (95% CI)		0.63 (0.41, 0.96)

P-value was based on log-rank test for serelaxin vs. placebo
Hazard ratio < 1.0 favors serelaxin

Finally, when a pooled analysis of RELAX-AHF and Pre-RELAX-AHF studies was performed, significantly fewer 180-day CV deaths in patients treated with serelaxin 30 µg/kg/day were observed when compared to placebo (K-M estimates of 5.6% vs. 9.8%). The hazard ratio using a Cox model was 0.56 (95% CI: 0.37-0.86) stratified by study and 0.57 (95% CI: 0.37-0.86) stratified by region.

8 Safety

8.1 Safety assessments

Safety was assessed by comparing the serelaxin group to the placebo in terms of the rate, type, severity and drug relationship of adverse events (AEs), deaths, serious adverse events (SAEs) and other clinically significant AEs, physical examination findings, AHF related signs and symptoms, changes in clinical laboratory parameters, effects on vital signs, and ECGs in a subset of patients evaluations. Specific assessments of neurohormonal or other markers of HF and renal function were also performed.

Based on the benign safety profile experienced in the Phase II dose-finding study Pre-RELAX-AHF, the Agency agreed that non-serious AEs were to be reported through Day 5 and serious adverse events (SAEs) through Day 14 in study RELAX-AHF. In fact, this AE collection window was consistent with other clinical effects observed over the first 5 and 14 days, respectively, and was long enough to both cover an interval 5 times the half-life of serelaxin after a 48-hour infusion and span the hospitalization time of the index event for the vast majority of patients (mean length of initial hospital stay: 10.1 days, 95% CI: 9.5 to 10.6 days). Deaths were captured as fatal SAEs through Day 14 and as a safety endpoint through Day 180.

Due to the vasodilatory properties of serelaxin, safety measures to manage confirmed blood pressure decrease events (CBPDEs) were proactively included in the study protocol RELAX-AHF. BP was to be monitored periodically during the 48-hour treatment with study drug. If a pre-specified systolic BP drop was confirmed by two consecutive measurements 15 minutes apart, the dose was adjusted or study drug infusion to be stopped and the event was to be documented as a CBPDE. If at any time during dosing, the patient's systolic BP was decreased by >40 mmHg from baseline but was still >100 mmHg, the study drug treatment infusion rate was decreased by 50% for the remainder of the study drug administration. The study drug infusion was terminated if at any time during dosing, the patient's systolic BP was <100 mmHg.

8.2 Adverse events

All treatment-emergent AEs, i.e., non-serious AEs through Day 5, SAEs through Day 14, were reported for a total of 56.1% of patients on placebo and 53.7% of patients treated with serelaxin. A total of 3.9% on placebo and 4.6% of patients on serelaxin discontinued due to an AE. SAEs and SAEs leading to discontinuation through Day 14 (Table 8-1) occurred in 13.7% and 0.5% of patients receiving placebo and in 15.1% and 0.9% of those receiving serelaxin, respectively.

Table 8-1 Overview of incidence of adverse events through Day 14 – RELAX-AHF (safety set)

Event	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Subjects With any AE	320 (56.1)	305 (53.7)
Subjects With Any Drug-Related AE[1]	46 (8.1)	47 (8.3)
Subjects with Any AE Leading to Study Drug Discontinuation	22 (3.9)	26 (4.6)
Subjects with any SAE	78 (13.7)	86 (15.1)
Subjects with any Drug-Related SAEs[1]	2 (0.4)	3 (0.5)
Subjects with Any SAE Leading to Drug Discontinuation	3 (0.5)	5 (0.9)
Serious AE With an Outcome of Death	15 (2.6)	10 (1.8)

Note: Incident AEs are considered those AEs with an onset date and time after the initiation of study drug.

[1] AEs assessed as definite, probable, or possible are considered drug-related.

All-treatment emergent AEs through Day 14

Due to the different collection periods of non-serious and serious AEs, an analysis of all AEs by SOC through Day 5 was performed (Table 8-2). As expected for this study population with AHF, the majority of AEs were observed in the System Organ Classes (SOCs) Cardiac disorders (placebo: 15.8%; serelaxin: 12.3%) with Cardiac failure congestive (placebo: 5.6%; serelaxin: 3.3%), Cardiac failure (placebo: 1.6%; serelaxin 0.7%) and Ventricular tachycardia (placebo: 1.8%; serelaxin 0.7%) as the most frequently reported AEs in this SOC.

Broadly, the AE profile by SOC and Preferred Terms (PT) was comparable between serelaxin and placebo. The majority of observed PTs reflected the underlying conditions of AHF and concomitant diseases.

Table 8-2 Incidence of adverse events by System Organ Class from study drug initiation through Day 5 - RELAX-AHF (safety set)

System Organ Class	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Subjects with at least one AE	305 (53.5)	280 (49.3)
Total number of AEs	703	539
Blood and lymphatic system disorders	6 (1.1)	10 (1.8)
Cardiac disorders	90 (15.8)	70 (12.3)
Ear and labyrinth disorders	3 (0.5)	2 (0.4)
Endocrine disorders	3 (0.5)	1 (0.2)
Eye disorders	1 (0.2)	3 (0.5)
Gastrointestinal disorders	59 (10.4)	45 (7.9)
General disorders and administration site conditions	39 (6.8)	32 (5.6)
Hepatobiliary disorders	10 (1.8)	1 (0.2)
Infections and infestations	27 (4.7)	24 (4.2)
Injury, poisoning and procedural complications	0	3 (0.5)
Investigations	40 (7.0)	31 (5.5)
Metabolism and nutrition disorders	68 (11.9)	71 (12.5)
Musculoskeletal and connective tissue disorders	21 (3.7)	27 (4.8)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2 (0.4)	4 (0.7)
Nervous system disorders	45 (7.9)	30 (5.3)
Psychiatric disorders	31 (5.4)	22 (3.9)
Renal and urinary disorders	32 (5.6)	26 (4.6)
Reproductive system and breast disorders	0	2 (0.4)
Respiratory, thoracic and mediastinal disorders	59 (10.4)	31 (5.5)
Skin and subcutaneous tissue disorders	2 (0.4)	7 (1.2)
Vascular disorders	41 (7.2)	29 (5.1)

Serious adverse events (SAEs) through Day 14

Overall, no clinically significant differences were observed in the SAE incidence rates between treatment groups. SAEs with an incidence rated of >0.5% by treatment group through Day 14 are presented in Table 8-3.

Table 8-3 Serious adverse events with an incidence rate of more than 0.5% from study drug initiation through Day 14 - RELAX-AHF (safety set)

Preferred Term	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Subject with any SAE	78 (13.7)	86 (15.1)
Total number of SAEs	107	105
Anaemia	3 (0.5)	0
Acute myocardial infarction	3 (0.5)	3 (0.5)
Bradyarrhythmia	4 (0.7)	2 (0.4)
Cardiac arrest	3 (0.5)	0
Cardiac failure acute	2 (0.4)	4 (0.7)
Cardiac failure congestive	12 (2.1)	10 (1.8)
Cardiac failure	3 (0.5)	7 (1.2)
Ventricular fibrillation	1 (0.2)	3 (0.5)
Pneumonia	5 (0.9)	1 (0.2)
Cerebrovascular accident	3 (0.5)	2 (0.4)
Renal failure	6 (1.1)	6 (1.1)
Acute pulmonary oedema	0	4 (0.7)

At each level of summation (system organ class and preferred term), subjects are counted once per SOC and PT. Incident AEs are considered those AEs with an onset date and time after the initiation of study drug.

There were 15 (2.6%) patients on placebo and 10 (1.8%) patients on serelaxin who experienced a fatal SAE (Table 8-4). None of the SAEs was assessed as suspected and unexpected (SUSAR), and there was no finding suggesting a significant safety risk to patients; thus, no expedited reporting occurred according to regulatory requirements.

Table 8-4 Incidence of serious adverse events with an outcome of death through Day 14 (safety set)

System Organ Class Preferred Term	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Subject with SAEs with an outcome of death	15 (2.6)	10 (1.8)
Total number of SAEs with an outcome of death	17	10
Cardiac disorders	8 (1.4)	4 (0.7)
Acute myocardial infarction	1 (0.2)	0
Cardiac arrest	2 (0.4)	0
Cardiac failure acute	0	1 (0.2)
Cardiac failure congestive	1 (0.2)	0
Cardiac failure	2 (0.4)	1 (0.2)
Cardiac tamponade	1 (0.2)	0
Cardiogenic shock	1 (0.2)	1 (0.2)
Dissecting coronary artery aneurysm	1 (0.2)	0
Pulseless electrical activity	0	1 (0.2)
Infections and infestations	1 (0.2)	1 (0.2)
Pneumonia	1 (0.2)	0
Sepsis	0	1 (0.2)
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	1 (0.2)	1 (0.2)
Lung cancer metastatic	1 (0.2)	0
Lung neoplasm	0	1 (0.2)
Nervous system disorders	3 (0.5)	2 (0.4)
Cerebrovascular accident	2 (0.4)	2 (0.4)
Embolic stroke	1 (0.2)	0
Renal and urinary disorders	1 (0.2)	1 (0.2)
Renal failure	1 (0.2)	1 (0.2)
Respiratory, thoracic and mediastinal disorders	2 (0.4)	1 (0.2)
Acute pulmonary oedema	0	1 (0.2)
Pulmonary haemorrhage	1 (0.2)	0
Respiratory failure	1 (0.2)	0

Note: At each level of summation (System Organ Class (SOC) and Preferred Term), subjects are counted once per SOC and Preferred Term. Incident AEs are considered those AEs with an onset date and time after the initiation of study drug.

Discontinuations

AEs leading to permanent discontinuation from study drug were reported by 26 (4.6%) patients in the serelaxin group and by 22 (3.9%) patients in the placebo group. According to primary SOC, the most frequently reported AEs leading to permanent discontinuation from study drug were vascular disorders (11 patients, 1.9% vs. 8 patients, 1.4%), nervous system disorders (5 patients, 0.9% in both groups), cardiac disorders (2 patients, 0.4% vs. 5 patients, 0.9%) and psychiatric disorders (4 patients, 0.7% vs. 0 patients) in the serelaxin group and placebo group, respectively. Hypotension (11 patients, 1.9% vs. 7 patients, 1.2%) was recorded as the most frequent AEs leading to permanent discontinuation from study drug in the serelaxin group and placebo group, respectively.

SAEs leading to drug discontinuation were reported by 5 (0.9%) patients in the serelaxin group and by 3 (0.5%) patients in the placebo group (Table 8-1).

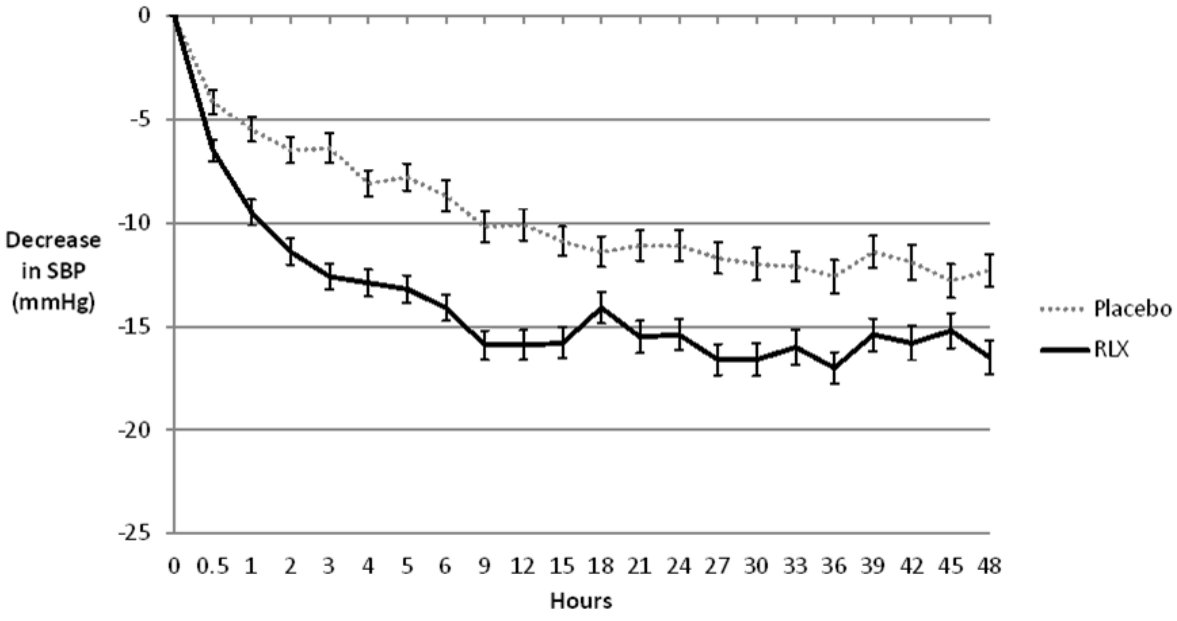
8.3 Vital signs and ECGs

Decreases in SBP, DBP from baseline were observed in both treatment groups, with more prominent decreases in SBP and DBP, respectively, in the serelaxin group (through Day 3 and 1, respectively) and greater decreases in heart rate as measured by pulse in the placebo group (through Hour 12).

As illustrated in Figure 8-1, a significantly greater decrease in systolic BP from baseline was observed for the serelaxin group compared to the placebo group during the 48 hours of continuous infusion. At the end of 24- and 48-hour infusion periods, systolic BP values were 128.7 ± 19.6 mmHg and 128.0 ± 17.3 mmHg in the serelaxin group, and 131.8 ± 17.8 mmHg and 131.0 ± 19.2 mmHg in the placebo group, respectively.

Hypotension and 'Confirmed blood pressure decrease events' (CBPDEs) are discussed in Section 8.5.1.

Figure 8-1 Changes in systolic BP from baseline during 48-hour infusion of 30 ug/kg/day serelaxin and placebo - RELAX-AHF (safety set)



Data on standardized ECGs collected in a substudy were available for 12 patients exposed to serelaxin in this study. The analysis of the QTcF intervals in these patients revealed no significant effect of serelaxin on cardiac repolarization or other ECG parameters. Interpretation of the results and conclusions drawn are limited due to the small sample size of the ECG substudy.

8.4 Laboratory parameters

Slight changes through Day 60 were noticed for variables such as RBC parameters, renal and hepatic events (see Section 8.5) in both treatment groups, all of which were assessed as not clinically significant in the setting of AHF.

8.5 Special safety topics

Based on the pharmacology of serelaxin and experience based on the development in the HF and non-HF indications, safety risks (hypotension, and transient decrease of hemoglobin/hematocrit) have been identified for serelaxin. Similar to other therapeutic proteins, serelaxin has the theoretical potential to induce hypersensitivity and is therefore closely monitored. In addition, safety topics have been analyzed that are in particular of special interest in the therapeutic area of AHF such as cardiac function including cardiac failure and arrhythmias, renal impairment, hepatic injury.

8.5.1 Hypotension

Hypotensive effects demonstrated in preclinical and clinical studies are consistent with the systemic vasodilatation mediated by serelaxin.

Confirmed blood pressure decrease events (CBPDEs)

Due to the vasodilatory properties of serelaxin, close BP monitoring was performed per study protocol. In case of 'Confirmed blood pressure decrease events' (CBPDEs), defined as SBP decrease by >40 mm Hg from baseline and/or to SBP <100 mm Hg at any time during study drug infusion with two consecutive measurements 15 minutes apart, dose reduction and discontinuation criteria were applied:

- if the SBP decreased by >40 mmHg from baseline but was >100 mmHg, study drug infusion rate was to be reduced by 50%;
- if the SBP was reduced to <100 mmHg, study drug infusion had to be permanently stopped;
- if the SBP further decreased <100 mmHg after initial 50% dose reduction, study drug infusion had to be permanently stopped.

Overall, more patients on serelaxin (167/568) than those on placebo (103/570) experienced a CBPDE (Table 8-5), which resulted in a 50% dose reduction only (patient could stay on drug) (serelaxin: 59/167; placebo: 31/103), 50% dose reduction with subsequent discontinuation (serelaxin: 16/167; placebo: 12/103), or immediate discontinuation of study drug without dose reduction (serelaxin: 91/167; placebo: 59/103).

In both treatment groups, the majority of CBPDEs occurred within the first 24 hours (median time to onset and 95% CI - serelaxin: 10.0 and 13.3-17.3 hours; placebo: 17.9 and 15.2-20.0 hours) were not accompanied by concomitant AEs and resolved without further intervention following dose reduction and/or discontinuation of the infusion (serelaxin: 141/167; placebo: 91/103). A total of 20 patients on serelaxin and 8 on placebo required treatment which consisted mainly of IV fluids; inotrope medication was provided to 2 patients on serelaxin and 3 patients on placebo.

Table 8-5 Subjects with CBPDEs, time to CBPDE and short-term outcome – RELAX-AF (safety set)

	Placebo (N=570)	Serelaxin (N=568)
Subjects with any CBPDE	103	167
50% dose reduction and patient stayed on drug	31	59
50% dose reduction followed by discontinuation	12	16
Discontinuation without dose reduction	59	91
Time to initial CBPDE; Median (hours)	17.9	10.0
Short-term outcome		
Resolved spontaneously	91	141
Required treatment (mostly IV fluids)	8	20
Not sufficient information	4	6

Among the serelaxin-treated patients with CBPDEs who completed the infusion on a reduced dose or were immediately discontinued, the systolic BP increased by approximately 10 mmHg during the first 60 minutes after dosing adjustment or discontinuation.

Within the serelaxin CBPDE subgroup, patients who discontinued study drug without dose reduction tended to have a lower SBP and ejection fraction (EF) in addition to a higher proportion of patients with NYHA class $\geq$III at baseline compared to patients who completed serelaxin infusion on a 50% reduced dose (mean SBP: 131.5 vs. 161.4 mmHg; mean EF %: 34.0 vs. 43.9; NYHA class III: 50% vs. 31.8%).

Notably, study protocol RELAX-AHF did not mandate to report CBPDEs as AE of Hypotension in contrast to Pre-RELAX-AHF. The overall incidence rate of the AE Hypotension or AEs indicative of hypotension through the first 5 days was in general low in both treatment groups (serelaxin: 4.9%; placebo: 4.6%). The AE hypotension was reported in 2.6% of patients treated with serelaxin and 1.6% of patients who received placebo (Table 8-6). No SAEs of hypotension occurred.

Table 8-6 Incidence of AEs potentially indicating an event of interest 'Hypotension', regardless of study drug relationship from study drug initiation through Day 5 – RELAX-AHF (safety set)

Preferred term	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Event of interest: Hypotension	26 (4.6)	28 (4.9)
Blood pressure decreased	2 (0.4)	3 (0.5)
Cardiogenic shock	1 (0.2)	0
Dizziness	11 (1.9)	7 (1.2)
Hypotension	9 (1.6)	15 (2.6)
Loss of consciousness	0	1 (0.2)
Orthostatic hypotension	0	1 (0.2)
Presyncope	1 (0.2)	0
Somnolence	0	1 (0.2)
Syncope	2 (0.4)	1 (0.2)

8.5.2 Transient decrease of hemoglobin, hematocrit and RBC

Anemia is common in CHF patients with an average prevalence around 40% (Silverberg et al 2006) and is recognized as an independent predictor of mortality (Groenveld et al 2008). Similarly, anemia has been found in up to 38.7% of AHF patients (Nieminen et al 2006; Oliva et al 2012). In AHF clinical trials, transient decreases in hemoglobin and hematocrit were associated with serelaxin treatment.

Hematology results in study RELAX-AHF

In study RELAX-AHF, minor decreases in hemoglobin were observed in the serelaxin group versus placebo with a mean difference from Baseline between serelaxin and placebo in the range of -0.3 mg/dL (through Day 3) and -0.1 (Day 4, Day 5); changes were statistically significant through Day 5. At Day 60, mean changes from baseline were -0.2 (1.35) g/dL in the serelaxin group and -0.1 (1.39) g/dL in placebo. These findings were mirrored by changes in hematocrit and RBC with a statistically significant decrease in the serelaxin group compared to placebo through Day 5. The mean difference between the treatment groups for hematocrit was in the range of -1%, for RBC in the range of -0.096 $x10^{12}$/L (through Day 3), -0.04 $x10^{12}$/L (through Day 4), -0.03 $x10^{12}$/L (Day 5). There was no change in MCV and MCH.

Overall, these observed hematology data are considered not clinically relevant. HF patients with low hemoglobin levels have significantly increased plasma volumes compared to those with normal hemoglobin, and hemodilution is a main contributing factor to anemia in this disease (Adlbrecht et al 2008).

AEs potentially indicative of hemoglobin/hematocrit transient decrease

Consistent with this interpretation, AEs potentially indicative of hemoglobin/hematocrit transient decrease occurred in an equal percentage of patients in the serelaxin and placebo groups through Day 5 (3.7% each) and Day 14 (4.4% each), in particular there were no signs of an increased bleeding risk with serelaxin treatment.

8.5.3 Events of interest in the indication AHF

8.5.3.1 Cardiac function including cardiac failure and arrhythmias

Patients treated with serelaxin experienced AEs associated with the SMQ ‘Cardiac failure,’ and the SMQs ‘Cardiac arrhythmias’ and ‘Torsade de pointes/QT prolongation’ less frequently through Day 14 (8.6% and 6.2%, respectively) compared to placebo (11.6% and 8.4%, respectively) (Table 8-7, Table 8-8).

Table 8-7 Incidence of AEs of the SMQ 'Cardiac failure' from study drug initiation through Day 14 - RELAX-AHF (safety set)

AEs of the SMQ cardiac failure	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Cardiac failure	66 (11.6)	49 (8.6)
Acute left ventricular failure	0 (0.0)	2 (0.4)
Acute pulmonary oedema	3 (0.5)	4 (0.7)
Cardiac asthma	0 (0.0)	1 (0.2)
Cardiac failure	11 (1.9)	8 (1.4)
Cardiac failure acute	6 (1.1)	6 (1.1)
Cardiac failure congestive	35 (6.1)	24 (4.2)
Cardiac resynchronisation therapy	1 (0.2)	0 (0.0)
Cardiogenic shock	1 (0.2)	1 (0.2)
Cardiorenal syndrome	1 (0.2)	0 (0.0)
Ejection fraction decreased	1 (0.2)	1 (0.2)
Hepatic congestion	3 (0.5)	0 (0.0)
Left ventricular failure	1 (0.2)	0 (0.0)
Oedema peripheral	4 (0.7)	1 (0.2)
Pulmonary congestion	1 (0.2)	0 (0.0)
Pulmonary oedema	2 (0.4)	1 (0.2)

Table 8-8 Incidence of AEs of the SMQs 'Cardiac arrhythmias' and 'Torsade de pointes/QT prolongation' from study drug initiation through Day 14 – RELAX-AHF (safety set)

AEs of the SMQs cardiac arrhythmias and Torsade de pointes/QT prolongation	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Subjects with at least one AE in either SMQ	**48 (8.4)**	**35 (6.2)**
Arrhythmia	5 (0.9)	2 (0.4)
Atrial fibrillation	2 (0.4)	4 (0.7)
Atrial flutter	4 (0.7)	3 (0.5)
Atrial tachycardia	3 (0.5)	3 (0.5)
Bradyarrhythmia	9 (1.6)	3 (0.5)
Cardiac arrest	3 (0.5)	0
Sinus bradycardia	0	5 (0.9)
Syncope	4 (0.7)	1 (0.2)
Tachycardia	3 (0.5)	0
Ventricular extrasystoles	3 (0.5)	3 (0.5)
Ventricular fibrillation	1 (0.2)	3 (0.5)
Ventricular tachycardia	10 (1.8)	4 (0.7)

Note: Presented AEs of ≥0.5% incidence in either treatment group only

8.5.3.2 Renal impairment

Treatment with IV infusion of serelaxin 30 µg/kg/day was associated with beneficial renal effects in patients with AHF. AEs indicative of renal impairment were reported in fewer patients in the serelaxin 30 µg/kg/day dose group (5.6%) compared to the placebo group (8.9%) through Day 14 (Table 8-9).

Table 8-9 Incidence of AEs potentially indicating an event of interest 'Renal impairment', regardless of study drug relationship from study drug initiation through Day 14 – RELAX-AHF (safety set)

SMQ 'Acute renal failure' Preferred term	Placebo (N=570)	Serelaxin (N=568)
Event of interest: Renal impairment	51 (8.9)	32 (5.6)
Azotemia	1 (0.2)	1 (0.2)
Blood creatinine increased	23 (4.0)	14 (2.5)
Oliguria	1 (0.2)	0
Proteinuria	2 (0.4)	0
Renal failure	25 (4.4)	14 (2.5)
Renal failure acute	0	2 (0.4)
Renal impairment	1 (0.2)	1 (0.2)

In general, mean serum creatinine and urea nitrogen (BUN) tended to increase in both treatment groups compared to baseline through Day 5; but the increases were more prominent in the placebo group and reached statistical significance compared to serelaxin. No differences were seen at Day 14 and Day 60. The categorical analysis of increased serum creatinine showed differences between treatment groups on Day 5 that were statistically significant for serum creatinine ≥0.5 mg/dL (serelaxin: 9.6%, placebo: 15.6%; p=0.003) (Table 8-10). No apparent differences were observed on Days 14 and 60.

Table 8-10 Creatinine increases from study drug initiation through Day 5 – RELAX-AHF (safety set)

Study day	Serum creatinine increase	Statistic	Placebo (N=570)	Serelaxin (N=568)	p-value
Day 5	≥0.3 mg/dl	n'	526	522	
		n (%)	147 (27.9)	119 (22.8)	0.055
	≥0.5 mg/dl	n'	526	522	
		n (%)	82 (15.6)	50 (9.6)	0.003
	≥1.0 mg/dl	n'	526	522	
		n (%)	15 (2.9)	10 (1.9)	0.321

n'= number of patients with measurement. P-values are based on Chi-Square test for serelaxin versus placebo.

8.5.3.3 Hepatic injury

Hepatic AEs were reported in fewer patients in the serelaxin 30 µg/kg/day dose group (0.9%) compared to the placebo group (2.8%) (Table 8-11).

Table 8-11 Incidence of AEs potentially indicating an event of interest 'Hepatic impairment', regardless of study drug relationship from study drug initiation through Day 14 – RELAX-AHF (safety set)

SMQ Drug related hepatic disorders - comprehensive search' Preferred Term	Placebo (N=570) n (%)	Serelaxin (N=568) n (%)
Event of interest: **Hepatic impairment**	16 (2.8)	5 (0.9)
Blood bilirubin increased	2 (0.4)	0
Cholestasis	1 (0.2)	0
Hepatic congestion	3 (0.5)	0
Hepatic cyst	1 (0.2)	0
Hepatic steatosis	1 (0.2)	0
Hyperbilirubinemia	2 (0.4)	0
Hypoalbuminemia	1 (0.2)	2 (0.4)
INR increased	4 (0.7)	3 (0.5)
Liver disorder	1 (0.2)	0

There were slight decreases in ALT and AST from baseline through Day 3 in both treatment groups and the changes were significantly greater for serelaxin compared to placebo.

Newly occurring elevations of liver function tests (LFTs) summarized by four different categories through Day 60 are presented in Table 8-12. There were no treatment differences in the proportion of patients in each category. Two patients (0.4%) in both treatment groups experienced ALT or AST >3 x ULN and total bilirubin >2 x ULN. As would be expected in this population, all four patients experienced LFT elevations in the setting of WHF or rehospitalization for AHF; most of these patients had hypotension or exhibited features of cardiogenic shock during the LFT increases.

Table 8-12 Newly occurring liver enzyme abnormalities at any time through Day 60 – RELAX-AHF (safety set)

Liver enzyme abnormality	Placebo (N=570) n/N (%)	Serelaxin (N=568) n/N (%)
ALT or AST >3xULN	17/555 (3.1)	13/555 (2.3)
ALT or AST >5xULN	5/562 (0.9)	6/563 (1.1)
ALT or AST >8xULN	4/564 (0.7)	1/565 (0.2)
ALT or AST >3xULN & TB >2x ULN	2/565 (0.4)	2/566 (0.4)

ULN: upper limit of normal

8.5.4 Hypersensitivity reactions including antibody formation

Similar to other therapeutic proteins, serelaxin has the theoretical potential to induce immunogenicity and cause hypersensitivity reactions, the latter of which may be independent of antibody formation. Based on the information currently available and consistent with the role of relaxin as an endogenous hormone released during repeat pregnancies, a significant safety risk to heart failure patients is not expected following repeat administrations of serelaxin.

8.5.4.1 AE indicative of hypersensitivity reactions

Overall across all HF trials, there were only sporadic reports of AEs that might have been indicative of immune system disorders or hypersensitivity/allergy. The majority of these AEs was of mild severity and not considered medically significant; no imbalance between serelaxin and placebo has been observed.

8.5.4.2 Antibody formation

One out of 559 patients tested across HF studies was anti-serelaxin antibody (Ab) positive (confirmed by the immunodepletion assay) on Day 30 post dose (study CRLX030A2201). However, the antibody titer was too low to be determined (in the titration assay undiluted sample generated signal lower than the screening cut point of the assay rendering no reportable titer) and no neutralizing activity was detected (in the cell-based neutralizing antibody assay). No AE indicating a hypersensitivity reaction was reported for this patient.

In contrast, sero-conversion was noted in 108 (43%) out of 251 patients, who were exposed to serelaxin in seven systemic sclerosis studies and for whom antibody (Ab) information is available. In those patients tested (n=42), antibodies were non-neutralizing using a bioassay *in vitro*. The likelihood of developing anti-serelaxin Abs was positively related to the duration of serelaxin exposure and to higher serelaxin dose, while no gender difference was observed. PK analysis indicated that presence of anti-serelaxin Abs was correlated with an increase in serum steady state concentration (Css) of serelaxin (1.8 to 3.7 fold median increase), likely due to a decreased clearance of serelaxin protein when bound to immunoglobulin (RLXN.C.003; RLXN.C.005). Ab formation was not associated with changes in BP, hemoglobin, osmolality, serum creatinine, predicted creatinine clearance, and uric acid (RLXN.C.005). However, all of these patients received continuous daily subcutaneous (sc) infusion of serelaxin for at least 2 weeks to approximately up to 48 weeks. This regimen is considered to be more immunogenic than the IV route of administration used in the HF population. In addition, the underlying autoimmune disease (Mehra et al 2013) might have stimulated Ab formation. In addition, there were no clinically significant hypersensitive reactions in any of the systemic sclerosis studies. Higher rates of injection site reactions >10% were observed across both serelaxin and placebo treatment groups and are believed to be mainly caused by the sc continuous infusion regimen for approximately up to 48 weeks in addition to the low pH 5 of the vehicle used for both the serelaxin and the placebo solution. In conclusion, Novartis considers the incidence rate of antibody formation as noted in systemic sclerosis studies unlikely to be representative of HF patients given the different mode of serelaxin administration and the longer duration of serelaxin dosing; furthermore no hypersensitivity reactions presenting a safety concern were reported. Across the entire serelaxin safety database, there were no reports indicating serelaxin-induced antoimmune disease.

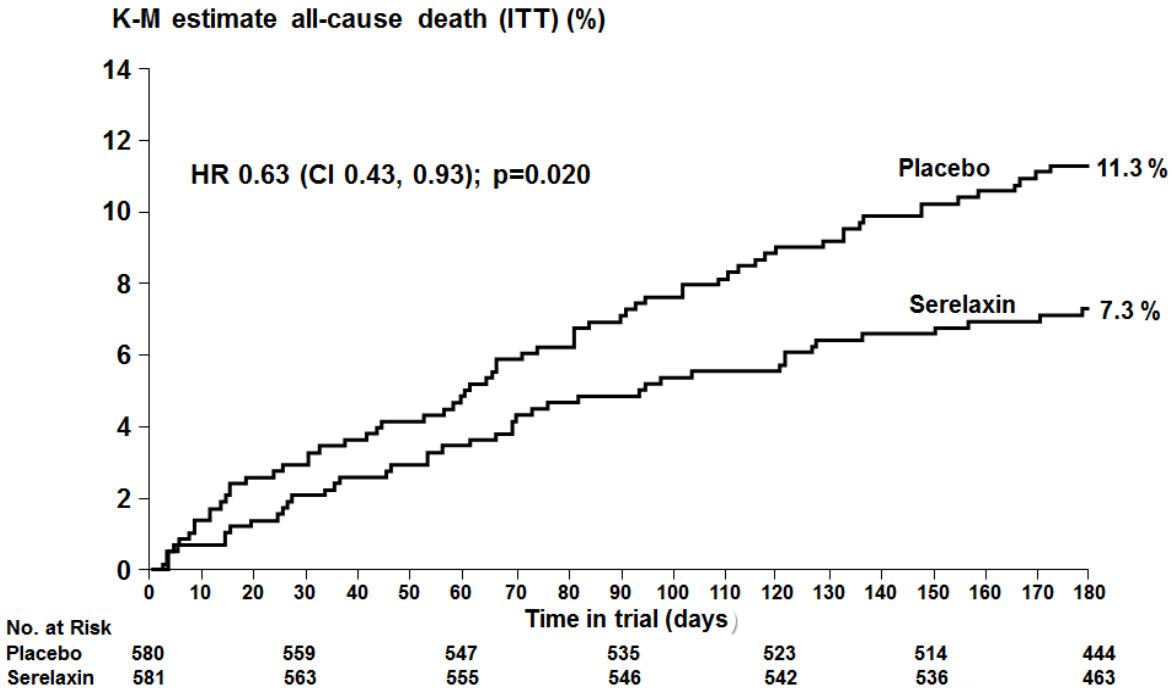
8.6 Long-term safety: all-cause mortality through Day 180

The all-cause mortality through Day 180 was a pre-specified safety endpoint in RELAX-AHF study. Results of all-cause mortality through Day 180 in the ITT set showed significantly fewer deaths (p=0.020) in patients treated with serelaxin, compared to placebo, with a total of 42 deaths (Kaplan-Meier estimate: 7.3%) in the serelaxin group vs. 65 deaths (Kaplan-Meier estimate: 11.3%) in the placebo group (Table 8-13, Figure 8-2). The hazard ratio was 0.63 (95% CI: 0.43, 0.93). Similar numbers were observed in the safety population showing 41 deaths with serelaxin (7.2%) and 64 with placebo (11.2%) (p <0.02; hazard ratio 0.63). Notably, the mortality difference was largely driven by the reduction of cardiovascular death through Day 180.

Table 8-13 All-cause mortality through Day 180 – RELAX-AHF (ITT set)

	Statistic	Placebo (N=580)	Serelaxin (N=581)
Number of events	n (%)	65 (11.2)	42 (7.2)
Kaplan-Meier estimates for time to death	Probability (95% CI)		
	Day 5	0.9 (0.4, 2.1)	0.7 (0.3, 1.8)
	Day 14	2.1 (1.2, 3.6)	1.0 (0.5, 2.3)
	Day 30	3.3 (2.1, 5.1)	2.1 (1.2, 3.6)
	Day 60	5.0 (3.5, 7.1)	3.5 (2.3, 5.3)
	Day 180	11.3 (9.0, 14.2)	7.3 (5.5, 9.8)
	p-value		0.020
Estimates by Cox model	Hazard Ratio (95% CI)		0.63 (0.43, 0.93)

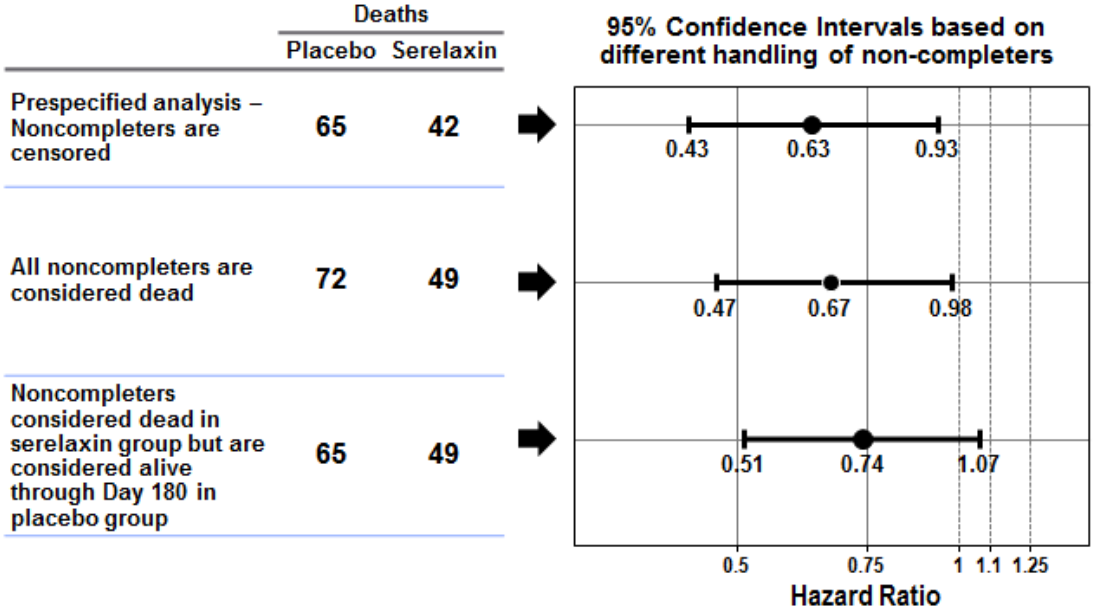
Figure 8-2 All-Cause Death through Day 180 – RELAX-AHF



Sensitivity analysis

Since the vital status at Day 180 could not be confirmed for a total of 14 patients in the RELAX-AHF study due to withdrawal consent or lost to follow-up (N=7 each in the serelaxin and placebo groups), a sensitivity analysis was performed, assuming that all 7 patients died in the serelaxin group (N=49) and all 7 patients in the placebo group were alive until Day 180 (N=65) from the time that they were lost to follow-up. The analysis demonstrated a HR of 0.74 (95% CI: 0.51-1.07), which provides persuasive evidence that serelaxin infusion was not associated with increased risk of death in RELAX-AHF study ($p<0.01$ for non-inferiority at a margin of 1.25) (Figure 8-3).

Figure 8-3 Evidence of no harm on all-cause mortality



Pooled analysis

In the pooled database of the Pre-RELAX-AHF and RELAX-AHF studies, the all-cause mortality rate at Day 180 was 7.4% in patients treated with serelaxin 30 µg/kg/day and 11.7% in patients that received placebo (HR=0.62; p=0.0128).

In summary, the results seen for all-cause mortality strongly support the conclusion that serelaxin is not associated with harm when administered along with the current standard of care.

9 Benefit/Risk

The aim of the serelaxin development program was to demonstrate that, in the target AHF patient population with high unmet medical need, serelaxin administered as a 48-hour IV infusion at 30 mg/kg/day provides significant symptom relief, as well as short-term (in-hospital) and long-term clinical benefits with a favorable safety profile.

The benefits of serelaxin were demonstrated in a pivotal Phase III study (RELAX-AHF) that randomized 1,161 patients hospitalized with AHF. In addition, positive trends in dyspnea relief and other clinical endpoints seen in the Phase II Pre-RELAX-AHF study were consistent with the results of the Phase III RELAX-AHF study. Hemodynamic studies also provided supportive mechanistic evidence.

The RELAX-AHF study patient population was representative of a 'real-world' AHF patient population. The Phase III RELAX-AHF study met its primary efficacy endpoint demonstrating that serelaxin treatment produced significant improvements in VAS AUC over a period of 5 days. This improvement was primarily driven by a reduction of in-hospital WHF events which represent a severe manifestation of AHF, prolonging the length of stay of the index hospitalization and is associated with a significantly increased risk of mortality. The serelaxin infusion was generally well-tolerated, and there was strong evidence of at least 'no harm' based on a decrease in all-cause mortality at 180 days following drug administration.

9.1 Summary of benefits

9.1.1 Short-term benefits

In the RELAX-AHF study, the 48-hour IV infusion of serelaxin in AHF patients was associated with significant benefits:

- Serelaxin treatment provided a clinically and statistically significant 19.4% improvement in the primary endpoint of dyspnea relief assessed by changes in VAS AUC from baseline through Day 5, with a mean treatment difference of 447.70 mm-hours vs. placebo (p=0.0075). The effect was consistent across multiple patient subgroups analyzed.
- This positive efficacy outcome was mainly driven by a 47% reduction in the WHF endpoint (largely representing in-hospital WHF events) through Day 5 (6.7% in serelaxin vs. 12.2% in placebo; HR=0.53, 95% CI: 0.36 to 0.79, p=0.0016). Fewer serelaxin patients experienced recurrent WHF events through Day 5. The reduction in risk of WHF was also maintained through Day 14 (11.4% vs. 15.7%; HR=0.70, 95% CI: 0.51 to 0.96, p=0.024).
- The improvement in dyspnea was associated with use of significantly lower doses of IV loop diuretics through Day 5 (161.3 mg in serelaxin vs. 213.0 mg in placebo, p=0.0057). In addition, patients on serelaxin treatment transitioned off IV diuretics sooner than the placebo patients through Day 5 (HR=1.24 favoring serelaxin, p=0.0001).
- Serelaxin improved physician-assessed HF signs and symptoms at the end of a 48-hour infusion ($p < 0.05$ vs. placebo).
- Serelaxin treatment was also associated with significantly lower NT-proBNP levels at Day 2, which is consistent with an improvement in cardiac wall stress.
- IV infusion of serelaxin produced rapid and sustained reduction in PCWP and PAP in hospitalized AHF patients (study CRLX030A2201), which provided mechanistic evidence supporting the improvement in patient-reported dyspnea.

- The other primary efficacy endpoint on the Likert scale (i.e., % of patients with moderate or marked improvement in dyspnea at 6, 12 and 24 hours) in RELAX-AHF did not demonstrate a treatment benefit. The discordant results between the VAS and Likert endpoints were due to the fact that the Likert scale did not capture the clinical deterioration and treatment failure such as in-hospital WHF events. When both improvement and worsening of dyspnea were analyzed post-hoc over a 5-day period from treatment initiation using the Likert scale, the results were consistent with the VAS AUC findings.

In addition, serelaxin treatment produced additional meaningful short-term in-hospital benefits:

- Significantly shorter length of stay for index hospitalization with a mean treatment difference of 0.9 days (9.6 days for serelaxin vs. 10.5 days for placebo, p=0.039).
- Significantly shorter ICU/CCU length of stay during the index hospitalization with a mean treatment difference of 0.3 days (3.5 days for serelaxin vs. 3.9 days for placebo, p=0.029).

Furthermore, the 48-hour IV infusion of serelaxin was associated with beneficial changes in both cardiac (hs-cTnT) and renal (cystatin-C) biomarkers, suggestive of reduced myocardial injury and renal dysfunction during the most vulnerable early period of exacerbation of AHF.

Taken together, serelaxin treatment in the Phase III RELAX-AHF study demonstrated numerous clinically important, short-term benefits for AHF patients that include improvement of dyspnea, prevention of in-hospital WHF events, less use of and faster transition off IV diuretics, and significantly shorter length of hospital stay. Similar effects consistent with the RELAX-AHF findings were also reported in the Phase II Pre-RELAX-AHF study. These results support a positive and clinically meaningful impact of serelaxin on the in-hospital clinical course of AHF patients.

9.1.2 Long-term effects

There have been no prior intervention studies in an AHF patient population demonstrating a long-term treatment benefit impacting mortality. In RELAX-AHF, serelaxin showed a potential long-term benefit on mortality:

- In RELAX-AHF, the two secondary efficacy endpoints, i.e., the composite of CV death and re-hospitalization due to HF/RF through Day 60, and DAOOH at Day 60 did not differ significantly in the serelaxin and placebo groups.
- For patients treated with serelaxin, a significant 37% relative risk reduction in CV mortality was observed through Day 180 (K-M estimate 6.1% for serelaxin vs. 9.6% for placebo, HR: 0.63, 95% CI: 0.41 to 0.96, p=0.028).
- This result is consistent with the reduction in CV mortality reported in the Phase II study Pre-RELAX-AHF.
- The beneficial changes in cardiac and renal biomarkers indicative of reduced myocardial injury and organ dysfunction provide a plausible mechanism for the potential long-term benefit of serelaxin on mortality.

9.2 Summary of risks

Overall, treatment with 48-hour IV infusion of serelaxin was well-tolerated. The risk of hypotension was manageable, and the use of serelaxin was also associated with a favorable renal profile. Specific safety observations and supporting studies included:

- The overall AE profiles were similar in both the serelaxin and placebo patients.

- BP decrease events can occur due to the vasodilatory properties of serelaxin. This safety risk is manageable with selection of the appropriate patients (i.e., SBP >125 mmHg prior to serelaxin treatment), careful BP monitoring, and dose reduction or discontinuation during serelaxin infusion as defined in RELAX-AHF.
- Serelaxin treatment was associated with beneficial renal effects in patients with AHF. Overall, AEs indicative of renal impairment were reported in fewer patients in the serelaxin group compared to the placebo group. In addition, significantly lower plasma cystatin-C values were observed at as earlier as Day 2 in serelaxin patients, and remained persistently lower than placebo patients through Days 5 and 14. A similar reduction in serum creatinine was also observed through Day 5. The incidence of worsening renal function at Day 2 was also lower in serelaxin compared to placebo patients. This favorable renal effect may be related to the increase in creatinine clearance observed in AHF patients (study CRLX030A2201).

Finally, the all-cause mortality data through Day 180 in RELAX-AHF support the favorable long-term safety profile of serelaxin, providing persuasive evidence of "no harm":

- Treatment with serelaxin was associated with a 37% relative risk reduction in all-cause mortality through Day 180 (7.3% in serelaxin vs. 11.3% in placebo; HR: 0.63, 95% CI: 0.43 to 0.93, p=0.020; ITT set).
- Since the vital status at Day 180 could not be confirmed for a total of 14 patients in the RELAX-AHF study due to withdrawal consent or lost to follow-up (N=7 each in the serelaxin and placebo groups), a sensitivity analysis was performed assuming that all 7 patients died in the serelaxin group and all 7 patients in the placebo group were considered alive until Day 180 from the time patients were lost to follow-up. The analysis demonstrated a HR of 0.74 (95% CI: 0.51-1.07).

9.3 Conclusion on benefit and risk

The RELAX-AHF program enrolled patients representative of the US AHF patient population. The totality of evidence obtained from RELAX-AHF, Pre-RELAX-AHF and the hemodynamic studies provides strong support for the efficacy and safety of serelaxin for the treatment of AHF, providing patients improvement of symptoms through significant reduction of WHF. Serelaxin is the first treatment that has demonstrated a clinically-relevant effect of preventing in-hospital WHF events without adversely effecting mortality. There was also improvement in physician-assessed HF signs and symptoms, less need for IV diuretics and faster transition off IV diuretics, and shorter length of hospital stay in the serelaxin-treated patients compared to placebo. The BP decrease associated with serelaxin treatment was manageable.

There was a significant reduction in cardiovascular and all-cause mortality observed with serelaxin through 180 days in RELAX-AHF, with an upper bound of the 95% CI for mortality <1.25 (under the most conservative assumptions for patients with missing data) that provides strong and persuasive evidence of no harm.

In conclusion, serelaxin has demonstrated a positive benefit-risk ratio through the overall results from the Phase III RELAX-AHF study, in conjunction with the Phase II Pre-RELAX-AHF and other studies, providing the basis for approval of serelaxin for the improvement of the symptoms of AHF through reduction in the rate of WHF.

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