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RESEARCH ARTICLE

Effects of serelaxin in patients admitted for acute heart failure: a meta-analysis

John R. Teerlink[1], Beth A. Davison[2], Gad Cotter[2]*, Aldo P. Maggioni[3], Naoki Sato[4], Ovidiu Chioncel[5], Georg Ertl[6], G. Michael Felker[7], Gerasimos Filippatos[8], Barry H. Greenberg[9], Peter S. Pang[10], Piotr Ponikowski[11], Christopher Edwards[2], Stefanie Senger[2], Sam L. Teichman[12], Olav Wendelboe Nielsen[13], Adriaan A. Voors[14], and Marco Metra[15]

[1]Section of Cardiology, San Francisco Veterans Affairs Medical Center and School of Medicine, University of California San Francisco, San Francisco, CA, USA; [2]Momentum Research, Inc., Durham, NC, USA; [3]Italian Association of Hospital Cardiologists (ANMCO) Research Center, Florence, Italy; [4]Cardiology and Intensive Care Unit, Nippon Medical School, Musashi-Kosugi Hospital, Kawasaki, Japan; [5]University of Medicine Carol Davila, Bucharest, Romania; [6]Julius-Maximilians-Universität Würzburg, Würzburg, Germany; [7]Division of Cardiology, Duke University School of Medicine, Durham, NC, USA; [8]Athens University Hospital Attikon, Athens, Greece; [9]Division of Cardiology, University of California, San Diego, CA, USA; [10]Department of Emergency Medicine, Indiana University School of Medicine, Indianapolis, IN, USA; [11]Department of Heart Diseases, Medical University, Military Hospital, Wroclaw, Poland; [12]Formerly Corthera, San Carlos, CA, USA; [13]Department of Cardiology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark; [14]University Medical Center Groningen, Groningen, The Netherlands; and [15]Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia, Italy

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Aims The effectiveness and safety of 48 h intravenous 30 μg/kg/day serelaxin infusion in acute heart failure (AHF) has been studied in six randomized, controlled clinical trials.

Methods and results We conducted a fixed-effect meta-analysis including all studies of intravenous serelaxin initiated within the first 16 h of admission for AHF. Endpoints considered were the primary and secondary endpoints examined in the serelaxin phase III studies. In six randomized controlled trials, 6105 total patients were randomized to receive intravenous serelaxin 30 μg/kg/day and 5254 patients to control. Worsening heart failure to day 5 occurred in 6.0% and 8.1% of patients randomized to serelaxin and control, respectively (hazard ratio 0.77, 95% confidence interval 0.67–0.89; $P = 0.0002$). Serelaxin had no statistically significant effect on length of stay, or cardiovascular death, or heart or renal failure rehospitalization. Serelaxin administration resulted in statistically significant improvement in markers of renal function and reductions in both N-terminal pro-B-type natriuretic peptide and troponin. No significant adverse outcomes were noted with serelaxin. Through the last follow-up, which occurred at an average of 4.5 months (1–6 months), serelaxin administration was associated with a reduction in all-cause mortality, with an estimated hazard ratio of 0.87 (95% confidence interval 0.77–0.98; $P = 0.0261$).

Conclusions Administration of intravenous serelaxin to patients admitted for AHF was associated with a highly significant reduction in the risk of 5-day worsening heart failure and in changes in renal function markers, but not length of stay, or cardiovascular death, or heart or renal failure rehospitalization. Serelaxin administration was safe and associated with a significant reduction in all-cause mortality.

Keywords Vasodilators • Acute heart failure • Mortality

*Corresponding author. Momentum Research, Inc., 807 East Main Street, Building 6, Suite 6-050, Durham, NC 27701, USA. Tel: +1 919 937-9157, Email: gadcotter@momentum-research.com

Introduction

Although some studies in acute heart failure (AHF) over the last three decades have shown limited benefit with regard to symptoms[1] and weight reduction,[2] such studies have failed to identify new interventions that are both safe and efficacious in improving either short or long-term clinical outcomes.[3]

Serelaxin, a recombinant human relaxin-2, is a naturally occurring peptide. It produces many of the changes in cardiovascular (CV) and renal function observed during pregnancy and has end-organ protective, anti-fibrotic and anti-inflammatory effects in pre-clinical models. Previous work has demonstrated reductions in blood pressure and left ventricular filling pressure, pulmonary artery pressures and serum level of natriuretic peptides, as well as improvements in renal function and reduction in troponin release.[4–6] Serelaxin has been investigated in several prospective randomized studies in AHF.[7–14] Despite some subtle differences in study design, each study enrolled patients admitted for worsening symptoms and signs of heart failure (HF), with dyspnoea at rest or minimal exercise requiring additional therapy and who were randomized within 16 h of presentation to either treatment with intravenous serelaxin or a control group. Study drug was administered in addition to standard care. The control group was treated with matching placebo in all but one study, in which control patients were treated with standard care only. In the phase III studies, primary endpoints included worsening of heart failure (WHF), dyspnoea relief, and CV mortality. Only WHF was incorporated in the primary endpoints of all phase III studies. Secondary endpoints included length of initial hospital stay (LOS), the composite of CV death or HF or renal failure (RF) rehospitalization, and renal function. All-cause mortality was a key efficacy or safety outcome in all studies.

This is a post hoc meta-analysis, in which we estimated the effects of short-term intravenous infusion of serelaxin in adult patients admitted for AHF on pre-specified defined primary, secondary and safety endpoints of interest examined in the serelaxin phase III studies. In addition, we assessed changes in biomarkers defined prospectively in the RELAX-AHF study and a substudy of the RELAX-AHF-2 study.

Methods

We included all randomized controlled trials (RCTs) of intravenous serelaxin administered for 48 h in adult patients admitted for AHF. Clinical trial registries (ClinicalTrials.gov, EU Clinical Trials Register, World Health Organization International Clinical Trials Registry Platform) were searched for trials in HF associated with either serelaxin or relaxin as another term. PubMed was searched for studies of serelaxin in HF using the search '((heart failure[MeSH Major Topic] OR heart failure) AND clinical trial) AND (serelaxin OR relaxin)'. Studies in paediatric patients or in patients with chronic or compensated acute HF and mechanistic studies in which outcomes of interest were not measured or study drug was administered for a shorter period were excluded. The risk of bias for each included randomized study was assessed using Cochrane RoB 2.0.

For the six studies that were identified and met the above criteria, results comparing serelaxin 30 μg/kg/day with control were extracted from available data sources by one analyst and then verified by a second analyst. Sources included published manuscripts, public presentations, posted information on clinical trial registries,[7–13] results provided by the sponsor (Novartis Pharmaceutical Corporation), and clinical study reports (CSRs) prepared by the sponsor. CSR tables were available only for Pre-RELAX-AHF, RELAX-AHF, RELAX-AHF-ASIA and RELAX-AHF-2. Outcomes meta-analysed included WHF to day 5; CV mortality through day 180; LOS; CV death or HF/RF rehospitalization at last follow-up; changes from baseline to days 2, 5, and 14 in biomarkers including creatinine, cystatin C, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and high-sensitivity troponin; and all-cause mortality at last follow-up. We additionally analysed the 24 h change in systolic blood pressure.

Worsening of HF to day 5 was considered a dichotomous outcome and the serelaxin effect is expressed as a relative risk (RR). Hazard ratios (HRs) and associated standard errors were estimated from the available data for time-to-event outcomes.[14] Mean differences in continuous outcomes were estimated using reported means and standard deviations in the treatment groups. Mean changes and standard deviations for changes in biomarkers on the log scale were estimated by exponentiating reported geometric mean changes and 95% confidence intervals (CI).

Fixed-effect meta-analyses, using weighted least squares with inverse variance weighting, were conducted using the R package metafor.[15] Residual heterogeneity was estimated using a restricted maximum likelihood method.[16,17]

SAS® software version 9.4 (SAS Institute, Cary, NC, USA) was used for analyses except where otherwise noted. Two-sided $P < 0.05$ was considered statistically significant.

Results

Included studies

Of 19 randomized trials identified, 13 were excluded: two studies not in HF,[18,19] one paediatric study,[20] six studies in patients with chronic or compensated HF,[21–26] two haemodynamic studies in compensated AHF,[4,27] and two mechanistic echocardiographic studies[28,29] (online supplementary *Figure S1*). Six RCTs – two phase II (Pre-RELAX-AHF and RELAX-AHF-Japan) and four phase III trials – met the selection criteria and were incorporated into the meta-analysis (*Table 1*). In all these studies, serelaxin was administered to patients admitted for AHF in addition to standard care. Serelaxin 30 μg/kg/day administered as a continuous infusion for up to 48 h was studied in all of the RCTs. Additional serelaxin doses were studied in two phase II studies: 10, 100, and 250 μg/kg/day in Pre-RELAX-AHF and 10 μg/kg/day in RELAX-AHF-Japan. Because of the vasodilatory effects of serelaxin, all studies employed a nearly identical study drug dose adjustment algorithm based on systolic blood pressure monitoring. Five of the studies administered matching placebo to a control group in a double-blind manner; the sixth (RELAX-AHF-EU) was an open-label study in which the control group received standard care but no placebo. Thus, the risk of bias for assessing the effect of assignment to intervention was low for all but RELAX-AHF-EU.

Between October 2007 and June 2017, a total of 11 359 patients were enrolled in these six studies: 6105 patients to receive serelaxin 30 μg/kg/day and 5254 patients to the control arm. The

Table 1 Randomized controlled trials of intravenous infusion of serelaxin in adult patients admitted for acute heart failure and included in the meta-analysis

	Pre-RELAX-AHF	RELAX-AHF	RELAX-AHF-2	RELAX-AHF-Japan	RELAX-AHF-EU	RELAX-AHF-ASIA
Patients, *n*	234 total, 103 placebo +30 μg/kg/day serelaxin	1161 total	6545 total	46 total, 30 placebo +30 μg/kg/day serelaxin	2650 total	876 total
Study population	Patients ≥18 years within 16 h of presentation for AHF, with dyspnoea at rest or on minimal exertion, pulmonary congestion, SBP >125 mmHg, eGFR 30–75 mL/min/1.73 m^2, BNP ≥350 or NT-proBNP ≥1400 pg/mL who had received ≥40 mg i.v. furosemide or equivalent	Patients ≥18 years within 16 h of presentation for AHF, with dyspnoea at rest or on minimal exertion, pulmonary congestion, SBP >125 mmHg, eGFR 30–75 mL/min/1.73 m^2, BNP ≥350 or NT-proBNP ≥1400 pg/mL who had received ≥40 mg i.v. furosemide or equivalent	Patients ≥18 years within 16 h of presentation for AHF, with dyspnoea at rest or on minimal exertion, pulmonary congestion, SBP ≥125 mmHg, eGFR 25–75 mL/min/1.73 m^2, BNP ≥500 or NT-proBNP ≥2000 pg/mL (750 and 3000 if ≥75 or with AF) who had received ≥40 mg i.v. furosemide or equivalent	Patients ≥20 years within 16 h of presentation for AHF, with dyspnoea on minimal exertion, pulmonary congestion, SBP ≥125 mmHg, eGFR 25–75 mL/min/1.73 m^2, BNP ≥350 or NT-proBNP ≥1400 pg/mL who had received ≥40 mg i.v. furosemide or equivalent	Patients ≥18 years within 16 h of presentation for AHF, with dyspnoea, pulmonary congestion, SBP ≥125 mmHg, eGFR 25–75 mL/min/1.73 m^2, BNP ≥500 or NT-proBNP ≥2000 pg/mL who had received i.v. furosemide or equivalent	Patients ≥18 years within 16 h of presentation for AHF, with dyspnoea at rest or on minimal exertion, pulmonary congestion, SBP ≥125 mmHg, eGFR 25–75 mL/min/1.73 m^2, BNP ≥350 or NT-proBNP ≥1400 pg/mL who had received ≥40 mg i.v. furosemide or equivalent
Interventions	Placebo or 10, 30, 100, 250 μg/kg/day serelaxin i.v. infusion up to 48 h	Placebo or 30 μg/kg/day serelaxin i.v. infusion up to 48 h	Placebo or 30 μg/kg/day serelaxin i.v. infusion up to 48 h	Placebo or 10 or 30 μg/kg/day serelaxin i.v. infusion up to 48 h	SOC or 30 μg/kg/day serelaxin i.v. infusion up to 48 h	Placebo or 30 μg/kg/day serelaxin i.v. infusion up to 48 h
	Assigned 3:2:2:2:2	Assigned 1:1	Assigned 1:1	Assigned 1:1:1	Assigned 1:2	Assigned 1:1
Study drug adjustment rules	Infusion terminated if SBP decreased from baseline to <100 mmHg or by ≥40 mmHg in two successive measurements 15 min apart	Infusion rate halved if SBP decreased from baseline by ≥40 mmHg but SBP >100 mmHg; discontinued if SBP <100 mmHg or significant AE or lab abnormality	Infusion rate halved if SBP decreased from baseline by ≥40 mmHg but SBP ≥100 mmHg; discontinued if SBP <100 mmHg in two consecutive measurements 15 min apart or significant AE or lab abnormality	Infusion rate halved if SBP decreased from baseline by ≥40 mmHg but SBP >100 mmHg; discontinued if SBP <100 mmHg	Infusion rate halved if SBP decreased from baseline by ≥40 mmHg but SBP ≥100 mmHg; discontinued if SBP <100 mmHg in two consecutive measurements 15 min apart	Infusion rate halved if SBP decreased from baseline by ≥40 mmHg but SBP ≥100 mmHg; discontinued if SBP <100 mmHg in two consecutive measurements 15 min apart or significant AE or lab abnormality
Study design	Randomized, double-blind, parallel group, placebo-controlled	Randomized, double-blind, parallel group, placebo-controlled	Randomized, double-blind, parallel group, placebo-controlled	Randomized, double-blind, parallel group, placebo-controlled	Randomized, open-label, parallel group	Randomized, double-blind, parallel group, placebo-controlled
Outcomes	Dyspnoea relief (moderately/markedly better dyspnoea at 6, 12, 24 h; VAS AUC to day 5), WHF to day 5, renal impairment, LOS, days alive out of hospital to day 60, 180-day CV mortality	Primary: dyspnoea relief (moderately/markedly better dyspnoea at 6, 12, 24 h; VAS AUC to day 5) Secondary: days alive out of hospital to day 60, 60-day CV death or HF/RF readmission. Others included WHF to days 5 and 14, LOS. Safety:180-day mortality	Primary: 180-day CV death, WHF to day 5 Secondary: 180-day mortality, LOS, 180-day CV death or HF/RF rehospitalization	Primary: non-serious AEs through day 5, SAEs through day 14, PK measures Secondary: SBP AUC through 48 h and day 5, cardiorenal biomarker changes through day 14. Others included clinical composite (success/no change/failure) through day 5, moderate/marked improved dyspnoea, dyspnoea VAS AUC through day 5, WHF through day 5, LOS, CV and all-cause mortality through day 60	Primary: adjudicated WHF through day 5 Secondary: no improvement in HF through day 5, WHF through day 14, WRF through day 14, LOS	Primary: clinical composite (success = symptom relief at day 2/no change/failure = WHF through day 5) through day 5 Secondary: WHF through day 5, 180-day mortality, 180-day CV mortality. Others included time to moderate/marked improved dyspnoea and dyspnoea VAS AUC through day 5

Table 1 Continued

	Pre-RELAX-AHF	RELAX-AHF	RELAX-AHF-2	RELAX-AHF-Japan	RELAX-AHF-EU	RELAX-AHF-ASIA
WHF definition	Physician-determined assessment on the basis of worsening symptoms or signs of HF and the need for the addition or institution of i.v. medications or mechanical support to treat AHF	Investigator-reported worsening signs or symptoms of HF necessitating intensification of i.v. or mechanical HF treatment	Reported by the investigator through day 5 post-randomization, and defined as worsening signs and/or symptoms of HF that required an intensification of i.v. therapy for HF, or mechanical ventilatory, renal, or circulatory support. Such treatment could include the institution or up-titration of i.v. therapy with a diuretic, nitrate, or other medication for HF, or institution of mechanical support such as mechanical ventilation, ultrafiltration, haemodialysis, intra-aortic balloon pump, or ventricular assist device. This endpoint also included patients who died of any cause or were rehospitalized for HF in this 5-day period	Investigator-reported worsening signs and/or symptoms of HF that required a newly added or an intensification of i.v. therapy for HF (e.g. up-titration of i.v. furosemide, i.v. nitrates or any other SOC) or mechanical ventilatory, renal or circulatory support	Adjudicated WHF requiring rescue therapy, or all-cause death, through day 5	Adjudicated in-hospital worsening of signs and/or symptoms of HF requiring intensification of i.v. HF therapy or mechanical ventilation, renal/circulatory support, rehospitalization due to HF/RF, or death through day 5
Follow-up period	AEs and SAEs through day 30, rehospitalizations through day 60, vital status through day 180	AEs through day 5, SAEs through day 14, rehospitalizations through day 60, vital status through day 180	AEs through day 5, SAEs through day 14, rehospitalizations and vital status through day 180	AEs through day 5, SAEs through day 14, rehospitalizations and vital status through day 60	AEs through day 5, SAEs through day 14, rehospitalizations and vital status through day 30	AEs through day 5, SAEs through day 14, rehospitalizations and vital status through day 180
Timeframe	December 2007–October 2008	October 2009–September 2012	October 2013–February 2017	January 2014–August 2014	January 2014–April 2017	March 2014–June 2017

AE, adverse event; AF, atrial fibrillation; AHF, acute heart failure; AUC, area under the curve; BNP, B-type natriuretic peptide; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HF, heart failure; i.v., intravenous; LOS, length of stay; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PK, pharmacokinetic; RF, renal failure; SAE, serious adverse event; SBP, systolic blood pressure; SOC, standard of care; VAS, visual analogue scale; WHF, worsening of heart failure (note that all studies included death as a WHF event); WRF, worsening of renal function.

designs and/or results of these studies have been previously published, posted or presented.[7–14] Eligibility criteria were quite similar across the studies. All of these studies enrolled adult patients, within 16 h of presentation for AHF, with dyspnoea, radiologic evidence of pulmonary congestion, and normal to elevated systolic blood pressure who had received intravenous loop diuretic. All patients required to have an elevated natriuretic peptide level and mild to moderate renal impairment. Main eligibility criteria for the studies are presented in the online supplementary *Table S1*.

Worsening HF was defined identically across the studies – death or worsening symptoms and/or signs of HF requiring rescue therapy – although its ascertainment differed somewhat (*Table 1*). WHF was adjudicated in RELAX-AHF-EU and RELAX-AHF-ASIA but was investigator-reported in the other studies. Mortality was collected through day 180 in four trials (Pre-RELAX-AHF, RELAX-AHF, RELAX-AHF-2 and RELAX-AHF-ASIA), through 60 days in RELAX-AHF-Japan, and through day 30 in RELAX-AHF-EU. CV death or HF/RF rehospitalization was reported through day 60 in Pre-RELAX-AHF, RELAX-AHF, and RELAX-AHF-Japan, and through day 180 in RELAX-AHF-2 and RELAX-AHF-ASIA; cause of death and reason for rehospitalizations were adjudicated in RELAX-AHF and RELAX-AHF-2. Imputation rules for LOS varied somewhat across studies. For patients still in hospital at day 60, LOS in RELAX-AHF-Japan and RELAX-AHF-2 was censored at day 60, in RELAX-AHF was censored at day 61, and in RELAX-AHF-EU was censored at day 30. For in-hospital death, LOS was set to the maximum observed +1 day – which was 62, 61, 33, and 31 days in RELAX-AHF, RELAX-AHF-2, Pre-RELAX-AHF, and RELAX-AHF-EU, respectively. Otherwise, missing LOS was set to the average LOS in all studies except RELAX-AHF-EU where it was set to 30 days. Biomarkers such as troponin and NT-proBNP were measured centrally in substudies of RELAX-AHF-2 and RELAX-AHF-EU; creatinine was assayed locally in RELAX-AHF-2 and centrally in the other studies for which these results were available. High-sensitivity troponin I was measured in Pre-RELAX-AHF and high-sensitivity troponin T in other studies.

Patient characteristics

Aside from racial differences due to differing geographic coverage of the various studies, characteristics of patients enrolled in the six RCTs were remarkably similar (*Table 2*, and online supplementary *Table S2*). The study populations were elderly, about 60% male, with a mean left ventricular ejection fraction around 40% and about half the patients with left ventricular ejection fraction <40%; about half of the patients were hospitalized for HF within the last 12 months and over three-quarters of the patients had mild-to-moderate symptoms (New York Heart Association class II/III) prior to decompensation. Patients had multiple co-morbidities, almost 90% with a history of hypertension, about half with a history of atrial fibrillation, ischaemic heart disease, and diabetes mellitus. On presentation, these patients had similar mean systolic blood pressure (approximately 145 mmHg), heart rate, and respiratory rate. Despite slightly differing inclusion criteria, the baseline laboratory values were similar across the trials.

Effect of serelaxin on the primary endpoints examined in the phase III serelaxin studies

Primary efficacy endpoints of the phase III studies included measures of dyspnoea or symptom relief, WHF, and CV death. The pre-specified primary endpoint, dyspnoea relief, was statistically significant in the RELAX-AHF study ($P = 0{\cdot}007$),[9] and 5-day death or WHF was nominally significant in the RELAX-AHF-EU study ($P = 0.0172$).[12] Neither 180-day CV death nor 5-day death or WHF in the RELAX-AHF-2 study differed significantly between the serelaxin and placebo treatment groups.[10] The primary success/unchanged/failure endpoint of the administratively terminated RELAX-AHF-ASIA trial did not differ significantly between the serelaxin and placebo treatment groups ($P = 0.196$).[13]

Effect of serelaxin on worsening heart failure

Worsening HF to day 5 was reported in all six studies, which occurred in 6.0% of all patients randomized to receive serelaxin and 8.1% of patients randomized to control. The estimated RR for WHF was <1.0 in all six studies and statistically significantly <1.0 in four studies. Results of the fixed-effect meta-analysis showed that serelaxin reduced the occurrence of WHF by 23% (RR 0.77, 95% CI 0.67–0.89; $P = 0.0002$) (*Figure 1*). In a sensitivity analysis, excluding the RELAX-AHF-EU study that was open-label (although WHF was adjudicated by a blinded committee) resulted in only a slightly reduced estimate of serelaxin effect on WHF – from a RR of 0.77 to 0.79 (online supplementary *Figure S2A*) – while heterogeneity remained statistically significant ($P = 0.0327$). Heterogeneity of the result was only eliminated in a sensitivity analysis that excluded RELAX-AHF-2 (online supplementary *Figure S3A*) in which a large change in the estimated RR for WHF was observed (from 0.77 to 0.60) with a heterogeneity *P*-value of 0.5822.

Effect of serelaxin on dyspnoea

Dyspnoea relief, reported by the patient on either a 7-level Likert scale (from markedly worsened to markedly improved) or a 100-point dyspnoea visual analogue scale (VAS), has been studied in the serelaxin programme. In RELAX-AHF, serelaxin treatment significantly reduced the dyspnoea VAS area under the curve through day 5 endpoint (mean difference 448, 95% CI 120–775 mm × h; $P = 0.007$) but not the other primary endpoint of moderately or markedly better dyspnoea through 24 h (27% vs. 26%; $P = 0.70$) compared to placebo.

The effect of serelaxin on symptom relief was examined in RELAX-AHF-Japan and RELAX-AHF-ASIA using a trichotomous endpoint of success/no change/failure. Treatment success was defined as improvements in dyspnoea and physician-assessed HF signs and symptoms at day 2, while failure was defined as death or WHF through day 5. While the proportion with treatment success was increased and treatment failure decreased in the serelaxin-treated group in the phase II RELAX-AHF-Japan study, in the administratively terminated phase III RELAX-AHF-ASIA

Table 2 Characteristics of patients enrolled in randomized controlled trials of serelaxin in acute heart failure

	Pre-RELAX (*n* = 103)	RELAX-AHF (*n* = 1161)	RELAX-AHF-2 (*n* = 6545)	RELAX-AHF-Japan (*n* = 30)	RELAX-AHF-EU (*n* = 2650)	RELAX-AHF-ASIA (*n* = 870)
Age, years[a]	69.7 (9.62)	72.0 (11.24)	73.0 (11.20)	78.1 (10.66)	75.5 (10.2)	69.6 (14.1)
Male sex	58 (56.3%)	725 (62.4%)	3908 (59.7%)	22 (73.3%)	1507 (56.9%)	558 (64.1)
Time from presentation to randomization, h	8.4 (5.35)	7.9 (4.63)	8.13 (4.494)	8.2		
BMI, kg/m^2		29.3 (5.71)	29.8 (6.36)	24.4 (4.16)	29.6 (5.9)	25.6 (5.3)
Systolic BP, mmHg[a]	148.6 (19.98)	142.16 (16.593)	146.2 (16.70)	142.5 (14.56)	145.8 (17.0)	145.8 (17.2)
Diastolic BP, mmHg[b]	83.8 (11.57)	81.9 (13.7)	82.1 (14.08)		81.1 (13.6)	83.5 (15.9)
Heart rate, bpm	80.8 (15.95)	81.7 (15.7)	83.5 (17.05)	88.5 (19.03)	82.9 (17.0)	86.3 (17.9)
Respiratory rate, breaths/min[a,b]	23.1 (3.73)	22.2 (4.8)	21.9 (4.59)		22.5 (5.0)	21.9 (4.4)
LVEF, %	38.7 (13.21)	38.62 (14.584)	38.92 (13.816)	43.0 (15.61)	42.6 (14.1)	39.6 (15.7)
LVEF <40%	34 (47.9%)	598 (54.8%)	3180 (51.9%)			266 (53.8%)
Dyspnoea VAS, mm[b]	43.6 (19.31)	44.2 (19.98)				
Oedema[a]						
0	24 (23.3%)	244 (21.1%)	917 (14.9%)			
1+	31 (30.1%)	339 (29.4%)	1838 (30.0%)			
2+	33 (32.0%)	349 (30.2%)	2131 (34.7%)			
3+	15 (14.6%)	222 (19.2%)	1250 (20.4%)			
History of HF			4854 (74.2%)	19 (63.3%)	1955 (73.8%)	556 (64.1%)
Prior HF hospitalization	34 (33.0%)	397 (34.2%)	3338 (54.6%)	14 (46.7%)		382 (68.8%)
History of hypertension	88 (85.4%)	1006 (86.6%)	5875 (89.8%)	26 (86.7%)	2397 (90.4%)	
History of atrial fibrillation or flutter	44 (42.7%)	602 (51.9%)	2781 (42.5%)		1035 (58.9%)	
History of diabetes mellitus	52 (50.5%)	551 (47.5%)	3013 (46.1%)	17 (56.7%)	1189 (44.9%)	
History of IHD	74 (71.8%)	603 (51.9%)	3217 (49.2%)	15 (50.0%)	1375 (51.9%)	
History of mitral regurgitation	26 (25.2%)	361 (31.1%)	3390 (51.8%)	18 (60.0%)		
History of stroke or other cerebrovascular event[a]	21 (20.4%)	157 (13.5%)	1008 (15.4%)		429 (16.2%)	
History of CABG[b]	18 (17.5%)	211 (35.0%)	961 (14.7%)			
IV nitrates at randomization		81 (7.0%)	360 (5.5%)	2 (6.7%)	127 (4.8%)	
Creatinine, μmol/L[a]	118.8 (41.57)	116.5 (33.21)	120.2 (34.12)	98.5 (25.17)		
BUN, mg/dL	28.3 (11.67)	27.44 (11.26)	26.89 (12.1)	22.2 (5.98)		
Lymphocytes, %[a]	19.3 (9.43)		18.57 (9.78)			
Sodium, mmol/L[a]	140.6 (3.66)	140.8 (3.59)	139.46 (4.27)	141.7 (2.45)		
Haemoglobin, g/dL	13.1 (1.72)	12.8 (1.86)	12.67 (1.96)	11.5 (1.96)		
Albumin, g/dL[b]	4.01 (0.443)	4.02 (0.433)				
eGFR, mL/min/1.73 m^2	52.6 (15.76)	53.49 (13.030)	51.3 (14.40)	49.1 (13.31)	51.6 (15.0)	51.0 (14.3)
Troponin T, μg/L[a,b], geometric mean (95% CI)		0.035 (0.033–0.037)[c]				
BNP, pg/mL		1389.34 (1404.226)	1629.7 (2462.76)			1416.9 (1180.0)
NT-proBNP, pg/mL	3834.3 (5023.52)	4975.48 (4818.583)	7961.9 (6700.26)	6286.0 (4071.16)	6708.4 (5734.3)	8135.9 (7703.0)
NT-proBNP, pg/mL, geometric mean (95% CI)		5064.06 (4805.29–5336.76)[c]				

BMI, body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; BUN, blood urea nitrogen; CABG, coronary artery bypass graft; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; IHD, ischaemic heart disease; IV, intravenous; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VAS, visual analogue scale.

[a]Covariates included in prognostic model for 180-day all-cause mortality in RELAX-AHF (Metra *et al.* 2013)[5].

[b]Covariates prognostic of worsening HF by day 5 in the placebo arm of RELAX-AHF (unpublished).

[c]Geometric mean with 95% CI based on intention-to-treat population in biomarker substudy for RELAX-AHF.

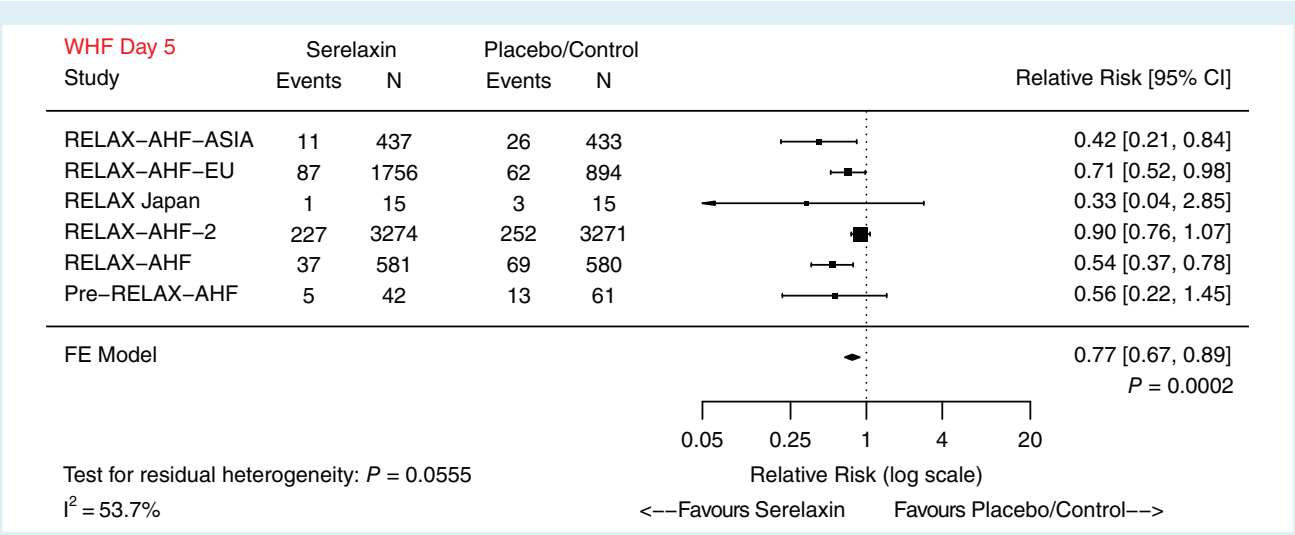

Figure 1 The effects of serelaxin on worsening heart failure (WHF) – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 11 359 total patients. CI, confidence interval.

trial, the proportion with treatment success was only nominally increased (19.7% vs. 19.2%) while the proportion with treatment failure was halved (4.1% vs. 8.3%) (odds ratio 0.82, 95% CI 0.60–1.11; $P = 0.196$).

The diversity of the methods used to assess dyspnoea and diverging imputation rules precluded us from performing a meta-analysis of the effects of serelaxin on dyspnoea.

Effect of serelaxin on cardiovascular mortality

The effect of serelaxin on CV mortality was available for four of the six studies. The 7% reduction in hazard estimated from these four studies in the fixed-effect meta-analysis was not statistically significant (HR 0.93, 95% CI 0.80–1.07; $P = 0.3109$) (*Figure 2*).

Effect of serelaxin on the secondary endpoints examined in the phase III serelaxin studies

Effect of serelaxin on length of stay

Length of stay was available for five studies. Serelaxin was not associated with a decrease in LOS (mean difference −0.09, 95% CI −0.42 to 0.24 days, $P = 0.5881$), and is presented in *Figure 3*.

Effect of serelaxin on the composite of cardiovascular death, or heart or renal failure rehospitalization

Effects on CV death or HF/RF rehospitalization were reported in five studies – through 60 days in Pre-RELAX-AHF, RELAX-AHF, and RELAX-AHF-Japan and through 180 days in RELAX-AHF-2 and RELAX-AHF-ASIA. Estimates from the meta-analysis (*Figure 4*) suggest that serelaxin does not affect the risk of this composite endpoint (HR 0.96, 95% CI 0.88–1.05; $P = 0.4324$).

Effect of serelaxin on kidney function

Reported mean changes in creatinine at days 2 and 5 were available for all studies. Serelaxin administration was associated with a statistically significant mean reduction in serum creatinine at both day 2 (mean difference −0.090, 95% CI −0.101 to −0.079 mg/dL; $P < 0.0001$) and day 5 (mean difference −0.065, 95% CI −0.080 to −0.049 mg/dL; $P < 0.0001$) (*Figure 5*).

Changes in cystatin C, which were available in three RCTs and in the biomarker substudies of RELAX-AHF-2 and RELAX-AHF-EU (online supplementary *Figure S4*), suggest that serelaxin had statistically significant beneficial effects on renal function at days 2, 5, and 14. Results in RELAX-AHF suggest that this effect may persist to day 60 from randomization.

Effect of serelaxin on mortality

No deaths were reported in the RELAX-AHF-Japan study; thus, this trial was excluded from the analysis of mortality. A total of 1042 patients died during follow-up, which occurred at a rough average of 4.5 months (1–6 months) across the five remaining studies. The effect of serelaxin on all-cause mortality estimated from the fixed-effect meta-analysis is presented in *Figure 6*. Serelaxin was associated with a statistically significant reduction in mortality risk with an estimated HR of 0.87 (95% CI 0.77–0.98; $P = 0.0261$) and non-statistically significant heterogeneity ($P = 0.2981$). In a sensitivity analysis excluding the RELAX-AHF-EU study which had shorter follow-up (online supplementary *Figure S2B*), the estimated HR for all-cause mortality was only slightly changed (to 0.88, 95% CI 0.78–1.00; $P = 0.0547$), suggesting that the effects of serelaxin are not due to differential follow-up in the treatment groups. A similar analysis excluding the

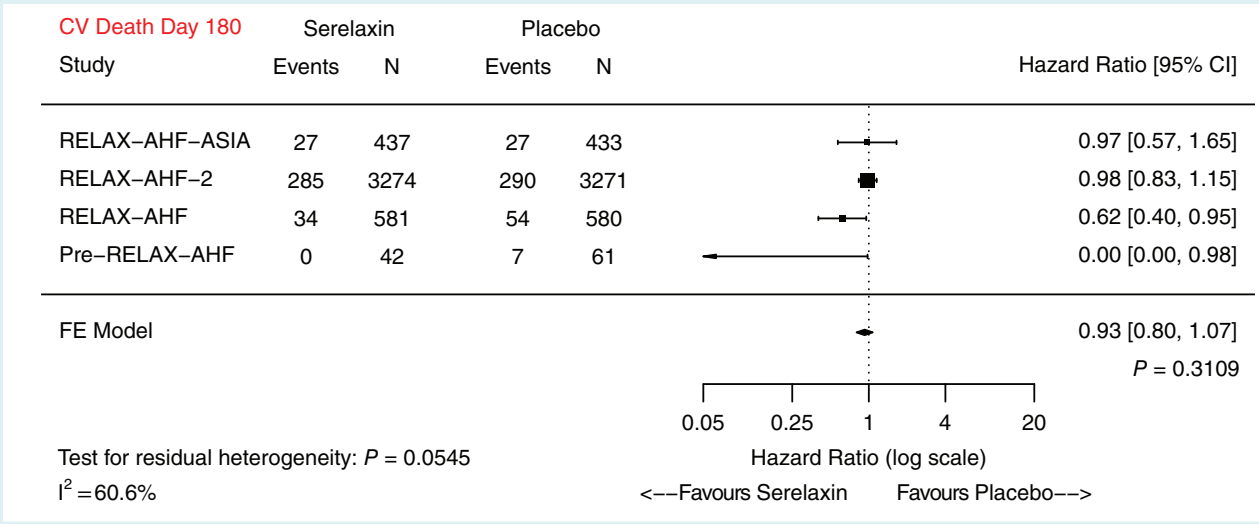

Figure 2 The effects of serelaxin on cardiovascular (CV) mortality – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 8679 total patients. CI, confidence interval.

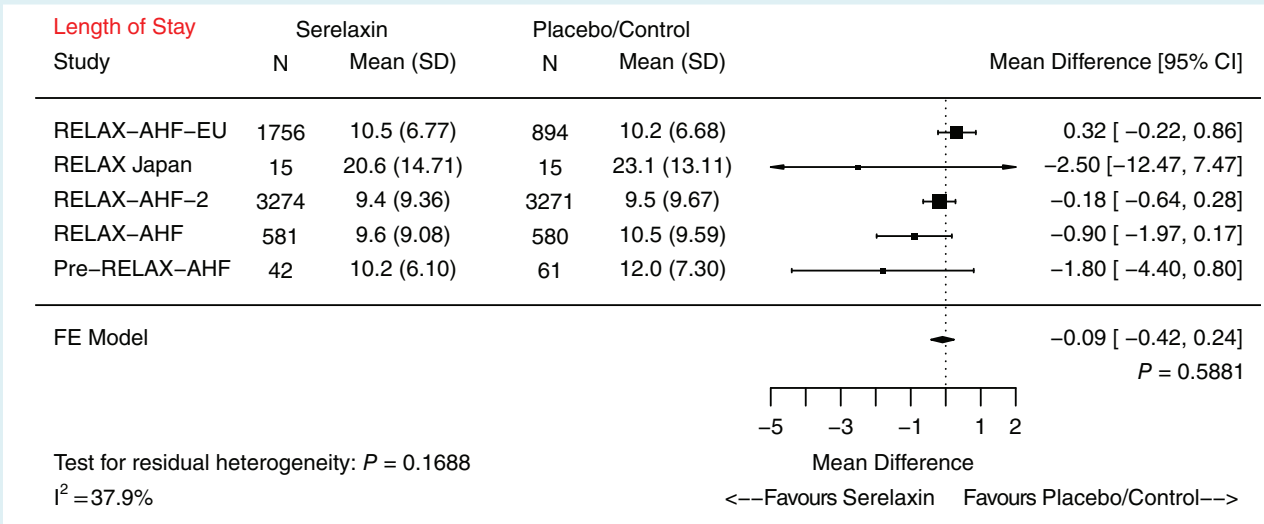

Figure 3 The effects of serelaxin on length of stay (days) – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 10 489 total patients. CI, confidence interval; SD, standard deviation.

RELAX-AHF-2 study (online supplementary *Figure S3B*) shows a marked change in the estimated HR (from 0.87 to 0.71).

Effect of serelaxin on other biomarkers

Serelaxin administration was associated with a reduction in the levels of NT-proBNP and troponin during the first 48 h while serelaxin was administered (*Figures 7* and *8*). At day 2, the treatment group ratio of relative changes from baseline in NT-proBNP was 0.86 (95% CI 0.81–0.90; $P < 0.0001$), and in troponin was 0.95 (95% CI 0.92–0.99; $P = 0.0060$). Although the effect on NT-proBNP decreased with time, the effect on troponin reduction (*Figure 8*) persisted and remained identical at day 14 (last follow-up).

Safety

Blood pressure changes during serelaxin administration were available for five of the six studies (online supplementary *Figure S5*). While systolic blood pressure decreased in all patients during the first 24 h, consistent with vasodilatory effects, blood pressure reductions were more pronounced in serelaxin-treated patients (mean difference −2.04, 95% CI −2.75 to −1.33 mmHg, $P < 0.0001$).

A summary of reported serelaxin safety data is presented in online supplementary *Table S3*. Adverse events (AEs) were collected through 30 days in the programme's first study and in all subsequent studies all AEs were collected through 5 days and only

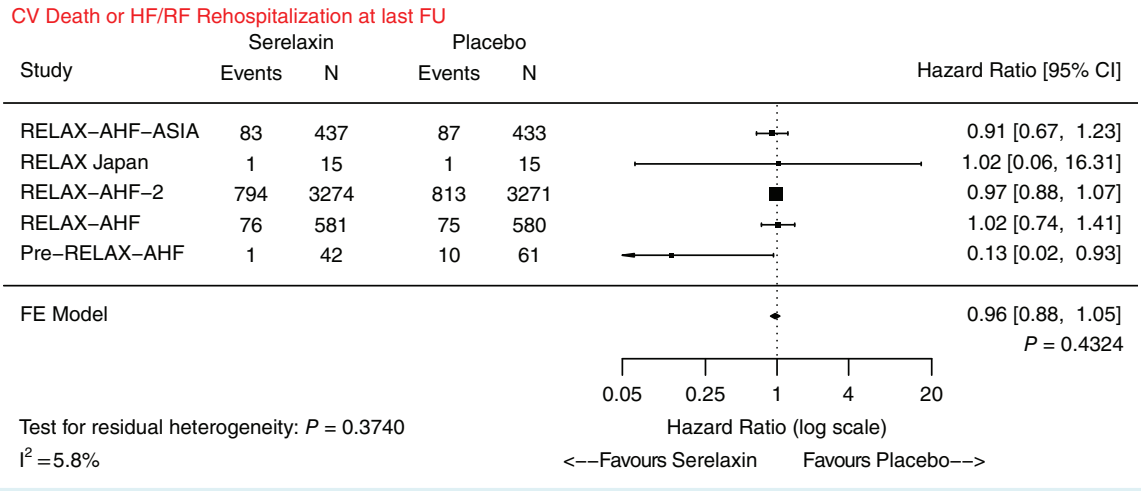

Figure 4 The effects of serelaxin on cardiovascular (CV) death or rehospitalization for heart failure (HF) or renal failure (RF) – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 8709 total patients. CI, confidence interval; FU, follow-up.

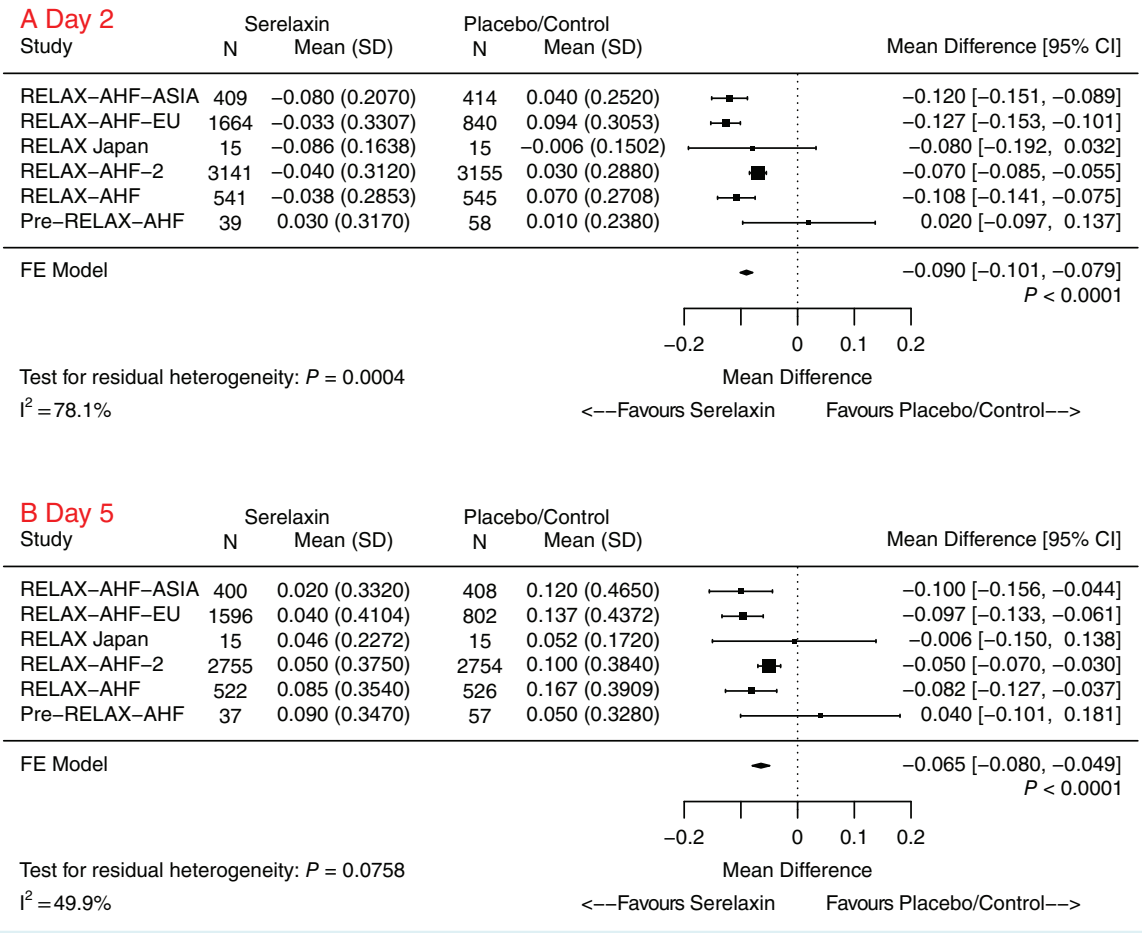

Figure 5 The effects of serelaxin on changes in creatinine (mg/dL) from baseline to *(A)* day 2 and *(B)* day 5 – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo; 10 836 total patients. CI, confidence interval; SD, standard deviation.

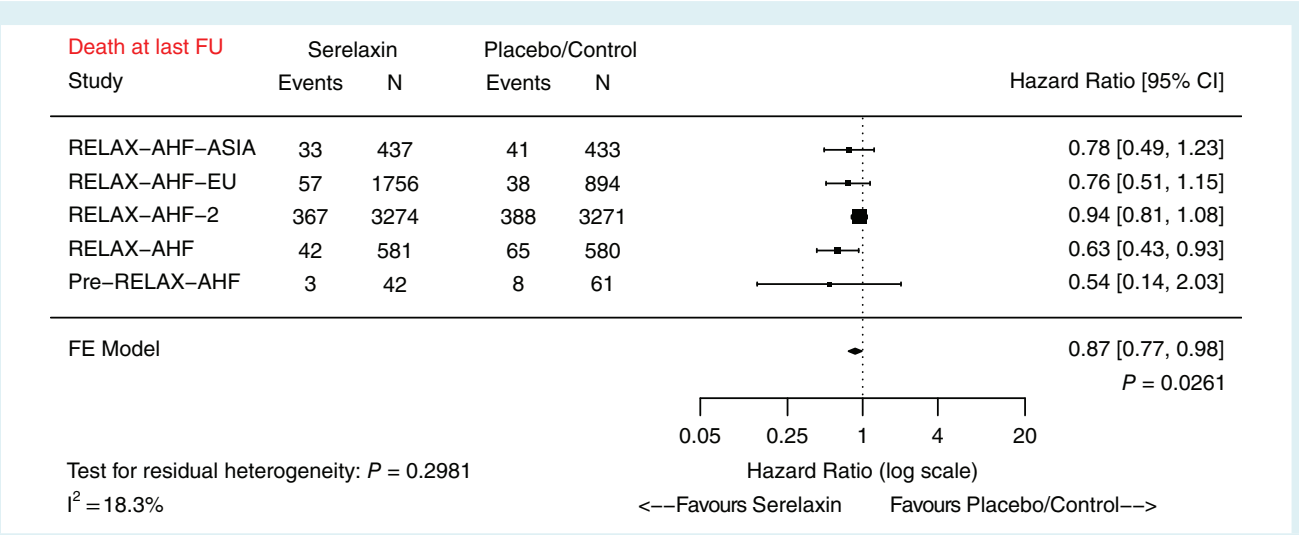

Figure 6 The effects of serelaxin on all-cause death at last follow-up (FU) – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 11 329 total patients. CI, confidence interval.

serious AEs collected after day 5 through day 14. Overall, intravenous infusion of serelaxin for up to 48 h was not associated with an increase in AEs, or serious AEs. Hypotension occurred more frequently in serelaxin-treated patients in each of the studies, along with a larger mean blood pressure decrease. Serelaxin-treated patients also experienced larger mean reductions in serum sodium and potassium levels, and haemoglobin levels. All of these were reported as being transient and resolved after study drug administration discontinuation.

Discussion

Serelaxin effects in adult patients with AHF were examined in six RCTs. To the authors' knowledge, these include all the RCTs conducted in the development of serelaxin for the treatment of AHF in adult patients. In these RCTs, the effects of serelaxin on in-hospital and short-term outcomes in patients admitted with AHF were examined in this meta-analysis. Of the primary endpoints examined in the serelaxin phase III studies, serelaxin had a highly statistically significant effect on WHF, but no significant effect on CV mortality was observed across the four studies reporting this outcome. Of the secondary endpoints examined in the serelaxin programme, serelaxin administration resulted in improvement in markers of renal function as measured by creatinine or cystatin C changes to at least day 5 but no statistically significant effect on the composite of CV death or HF/RF rehospitalization, or on LOS. Serelaxin administration was associated with a reduction of release and NT-proBNP during the time of study drug administration (first 2 days) and troponin to day 14, the last follow-up available. Finally, serelaxin was safe and associated with a reduction in all-cause mortality at last follow-up.

This meta-analysis is strengthened by the fact that the protocols for all presented studies were highly coordinated by a single sponsor (Corthera, a whole owned subsidiary of Novartis) and designed specifically to be largely identical with subtle variations, making the results of the meta-analysis more credible.

Worsening HF was assessed in all the identified RCTs of serelaxin in AHF, and was either the primary endpoint or a component of the primary endpoint of all the phase III studies. WHF was defined identically in all studies with slight variations in the way it was documented varying from a form completed by an investigator to fully blinded adjudication in RELAX-AHF-EU and RELAX-AHF-ASIA. This is of special importance in RELAX-AHF-EU where the study was not blinded. However, sensitivity analysis excluding RELAX-AHF-EU from the analysis of WHF showed virtually no change in the estimated RR for WHF (RR 0.79, $P = 0.0019$) and the P-value for heterogeneity remained statistically significant (0.0327) suggesting that the unblinded nature of RELAX-AHF-EU did not drive the evidence for a significant effect of serelaxin on WHF.

Importantly, serelaxin effect on mortality emerges in the context of its effect on decreasing troponin release which remained persistent to day 14, renal function and WHF, all of which were found to be highly associated with mortality.[5] However, serelaxin was also found to be associated with anti-fibrotic and anti-inflammatory effects in pre-clinical models,[4–6] which may contribute to some of its effects.

The totality of the data available from these six RCTs, including more than 11 000 patients in studies where serelaxin was initiated within the first 16 h of admission and administered with an identical dosing regimen for 48 h to AHF patients similarly characterized, would have been persuasive (and probably would have led to regulatory approval) if all of these patients had been recruited in a single large study. However, as it stands the effects of serelaxin were examined in six slightly differing studies with some variation in the pre-specified primary endpoints. The second largest (RELAX-AHF-EU) and third largest

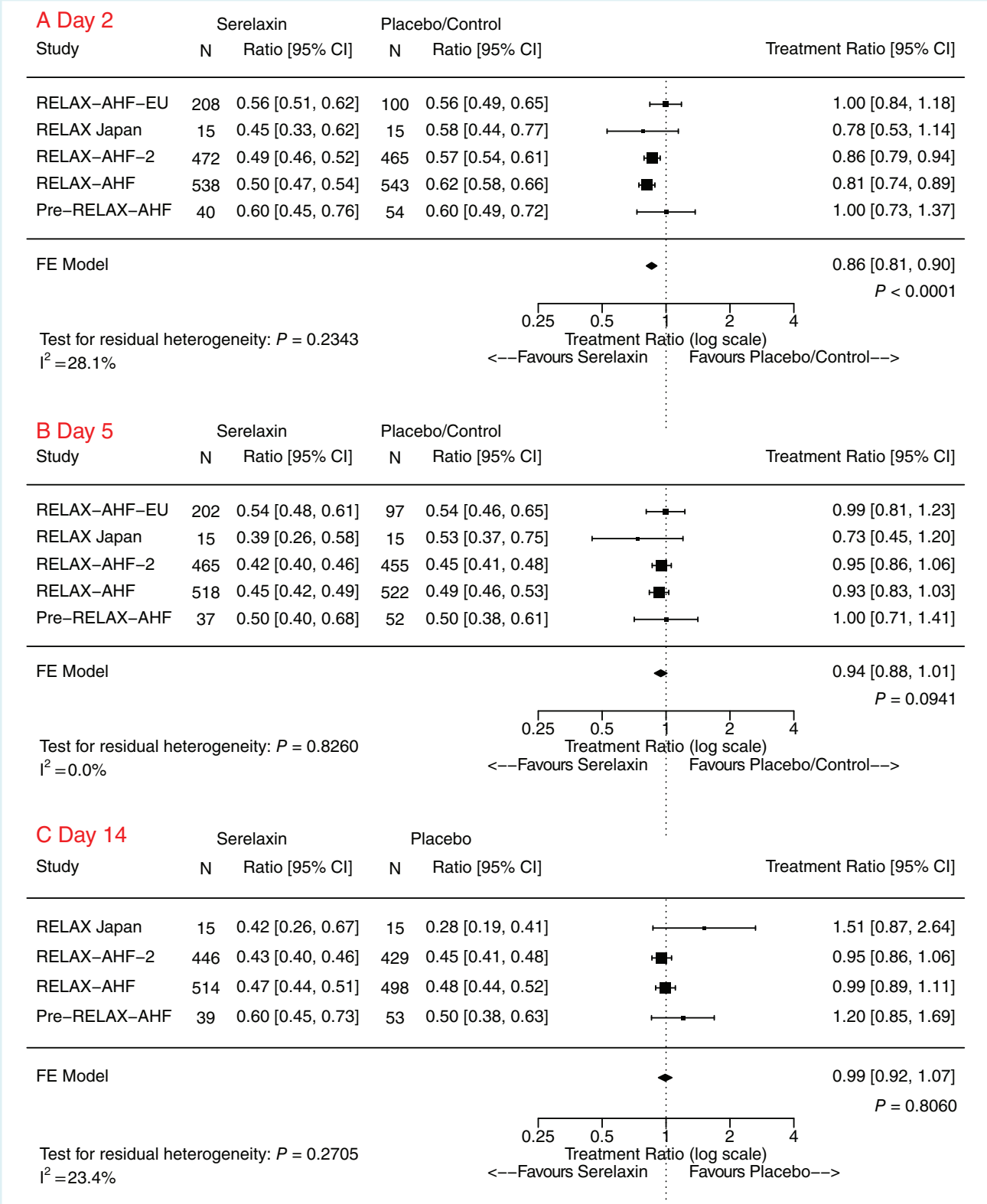

Figure 7 The effects of serelaxin on changes in N-terminal pro-B-type natriuretic peptide from baseline to *(A)* day 2, *(B)* day 5, and *(C)* day 14 – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 2450 total patients. CI, confidence interval.

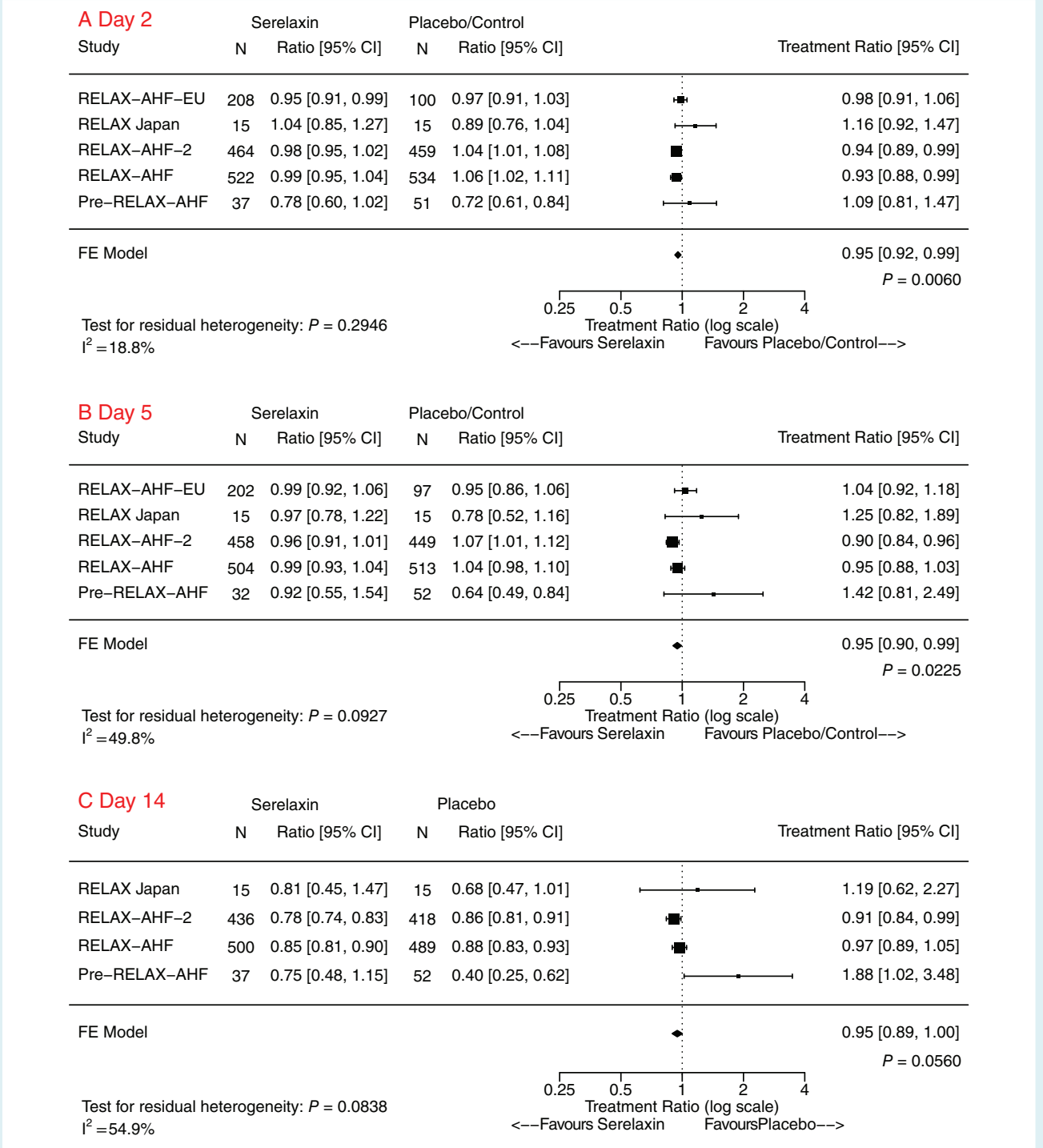

Figure 8 The effects of serelaxin on changes in troponin from baseline to *(A)* day 2, *(B)* day 5, and *(C)* day 14 – fixed-effect (FE) meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 2405 total patients. CI, confidence interval.

studies (RELAX-AHF) were positive as they met their primary endpoints. The fourth largest study (RELAX-AHF-ASIA) was terminated by the sponsor with only half the intended patients enrolled and hence was underpowered for the primary endpoint, but still showed a significant effect of serelaxin on WHF. Two of the studies were phase II studies and were not powered towards statistical significance on a clinical endpoint; however, in the fifth largest study (Pre-RELAX-AHF), WHF was significantly reduced. Moreover, in all six studies the effects of serelaxin on WHF and all-cause mortality were directionally similar suggesting

a benefit for serelaxin therapy. However, the effects of serelaxin were numerically smallest in the largest study (RELAX-AHF-2). In essence, the current meta-analysis shows that the data on serelaxin from six clinical studies can be largely divided into two equal parts from the perspective of number of patients enrolled. On the one hand, five studies including the original Pre-RELAX-AHF and RELAX-AHF studies and the chronologically last initiated three studies (RELAX-AHF-Japan, RELAX-AHF-EU and RELAX-AHF-ASIA) are homogeneous and suggest a clear benefit of serelaxin (online supplementary *Figure S3*). A number of potential reasons for such an occurrence may be proposed. First, the reduced effect in RELAX-AHF-2 could represent a chance finding. However, as can be seen in *Figures 1* and *6*, the CIs of all the studies included overlap and the point estimates for the HR from the meta-analysis is contained in the CIs of all the studies (i.e. none of the studies included rule out the possibility of up to a 24% reduction in WHF and a 19% reduction in all-cause death). Second, as highlighted by the authors in the manuscript describing the results of the RELAX-AHF-2 study, despite higher baseline natriuretic peptide levels and lower renal function measures as entry criteria in RELAX-AHF-2, the placebo event rates were actually lower than in RELAX-AHF, particularly for all-cause mortality and WHF.[10] This suggests that patients enrolled in RELAX-AHF-2 were possibly less sick and hence potentially less responsive to serelaxin therapy. Regardless, scientific evidence is strengthened through replication of the experiment. Although single large studies are acceptable for determining efficacy and allowing regulatory acceptance of new drugs, the regulators[30] have warned against the overreliance on single large studies for 'definitive' determination of efficacy. The example of the serelaxin programme where the overall data suggest benefit of serelaxin with regard to certain aspects of AHF in a large number of patients, despite smaller apparent effects in the largest study, should serve as a reminder of the strengths and weaknesses of larger studies vis-à-vis demonstrating efficacy in multiple studies.

Limitations

The current analysis is limited by the data available to the authors based on the data sources described above.

Conclusions

No current AHF therapy improves short or longer-term outcomes. This meta-analysis of the effects of serelaxin in AHF demonstrates that, in 11 359 patients enrolled in six prospective, largely similar RCTs, serelaxin administered to adult patients with AHF as a continuous intravenous infusion of 30 μg/kg/day initiated within the first 16 h of admission is safe and suggests that it is efficacious in producing highly significant reductions in WHF and markers of renal function and troponin while associated with a significant reduction in all-cause mortality.

Conflict of interest: J.R.T. reports grants, personal fees and non-financial support from Novartis, during the conduct of the study; grants, personal fees and non-financial support from Amgen and Trevena, outside the submitted work. B.A.D. reports personal fees from Novartis Pharma AG; grants from Novartis Pharmaceutical Corp, during the conduct of the study; grants from Amgen Inc., Celyad, Cirius Therapeutics Inc, Laguna Pharmaceuticals, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, Ventrix, outside the submitted work. G.C. reports personal fees from Novartis Pharma AG; grants from Novartis Pharmaceutical Corp, during the conduct of the study; grants from Amgen Inc., Celyad, Cirius Therapeutics Inc, Laguna Pharmaceuticals, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, Ventrix, outside the submitted work. A.P.M. reports personal fees from Bayer, Novartis, Fresenius, outside the submitted work. N.S. reports personal fees from Novartis, during the conduct of the study; personal fees from Otsuka, Ono, Terumo; grants from Daiichi-Sankyo, Roche Diagnostics Japan, outside the submitted work. O.C. reports grants from Servier, Vifor Pharma; grants and other from Novartis, outside the submitted work. G.E. reports grants from Bayer, Novartis, and Vifor Pharma, outside the submitted work. G.M.F. reports grants from Novartis, personal fees from Novartis, during the conduct of the study; personal fees from BMS, Medtronic, Roche Diagnostics, SC Pharma, Innolife, Abbott, Cardionomic, EBR Systems, LivaNova, grants and personal fees from Amgen, Cytokinetics, grants from Merck, outside the submitted work. G.F. reports being Steering Committee member for Novartis, during the conduct of the study; Steering Committee member for Medtronic, for BI, for Bayer, for Vifor Pharma, outside the submitted work. B.H.G. reports personal fees from Novartis, during the conduct of the study. P.S.P. reports grants, personal fees and non-financial support from Novartis, during the conduct of the study; grants, personal fees and non-financial support from Trevena, Roche, BMS, Baxter, grants from OrthoDiagnostics, NIH/NHLBI, AHRQ, AHA, outside the submitted work. P.P. reports personal fees and other from Novartis, during the conduct of the study; grants and other from Vifor Pharma, personal fees from Amgen, grants, personal fees and other from Bayer, BMS, Boehringer Ingelheim, Cardiorentis, Cibiem, grants from Singulaex, other from Fresenius, outside the submitted work. C.E. reports grants from Novartis Pharmaceutical Corp, during the conduct of the study; grants from Amgen Inc., Celyad, Cirius Therapeutics Inc, Laguna Pharmaceuticals, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, Ventrix, outside the submitted work. S.S. reports grants from Novartis Pharmaceutical Corp, during the conduct of the study; grants from Amgen Inc., Celyad, Cirius Therapeutics Inc, Laguna Pharmaceuticals, Sanofi, Roche Diagnostics Inc., Trevena Inc., NIH, Ventrix, outside the submitted work. S.L.T. reports other from Novartis, during the conduct of the study; other from Corthera, outside the submitted work; has a patent licensed to Novartis. O.W.N. reports personal fees from Novartis, during the conduct of the study. A.A.V. reports personal fees from Novartis, during the conduct of the study; personal fees from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Cytokinetics, GSK, Servier, Merck, grants and personal fees from Roche Diagnostics, outside the submitted work. M.M. reports personal fees from consulting honoraria from Novartis as Co-chairman of the RELAX-AHF-2 trial and the previous RELAX-AHF trials, during the conduct of the study; personal fees from consulting honoraria as Committees member or for speeches from Bayer, Fresenius, Servier, outside the submitted work.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Identification of studies included in the meta-analysis.

Figure S2. Sensitivity fixed-effect meta-analysis excluding RELAX-AHF-EU for the effects of serelaxin on (*A*) worsening heart failure to day 5, and (*B*) all-cause mortality.

Figure S3. Sensitivity fixed-effect meta-analysis excluding RELAX-AHF-2 for the effects of serelaxin on (*A*) worsening heart failure to day 5, and (*B*) all-cause mortality.

Figure S4. The effects of serelaxin on renal function: changes in cystatin C from baseline to days 2, 5, 14, and 60 – fixed-effect meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 2450 total patients.

Figure S5. Serelaxin effect on blood pressure change to 24 h – fixed-effect meta-analysis; serelaxin 30 μg/kg/day vs. placebo, 9685 total patients.

Table S1. Main inclusion and exclusion criteria of the major serelaxin studies.

Table S2. Characteristics of patients enrolled in randomized controlled trials of serelaxin in acute heart failure.

Table S3. Safety of 48 h intravenous infusion of serelaxin in randomized controlled studies.

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