

Effects of serelaxin in subgroups of patients with acute heart failure: results from RELAX-AHF

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Aim	Patients hospitalized for acute heart failure (AHF) differ with respect of many clinical characteristics which may influence their prognosis and response to treatment. We have assessed possible differences in the effects of serelaxin on dyspnoea relief, 60 Day outcomes and 180 Day mortality across patient subgroups in the RELAX-AHF trial.
Methods and results	Subgroups were based on pre-specified covariates (age, sex, race, geographic region, estimated glomerular filtration rate, time from presentation to randomization, baseline systolic blood pressure, history of diabetes, atrial fibrillation, ischaemic heart disease, cardiac devices, i.v. nitrates at randomization). Other covariates which may modify the efficacy of AHF treatment were also analysed. Subgroup analyses did not show any difference in the effects of serelaxin vs. placebo on dyspnoea relief or on the incidence of cardiovascular death or rehospitalizations for heart failure or renal failure at 60 days. Nominally significant interactions between some patient subgroups and the effects of serelaxin on 180 days cardiovascular and all-cause mortality were noted but should be interpreted cautiously due to the number of comparisons and the low incidence of deaths in the subgroups at lower risk.
Conclusion	The effects of serelaxin vs. placebo appeared to be similar across subgroups of patients in RELAX-AHF.
Keywords	Acute heart failure • Serelaxin • Mortality • Subgroups

Introduction

Heart failure (HF) is the most important cause of hospitalization for subjects older than 65 years and these hospitalizations are associated with high mortality rates, up to 10-20% in the 6 months following discharge, and 5-15-fold higher than those of ambulatory patients with chronic HF.¹⁻³ However, treatment of acute HF (AHF) has not changed in the recent decades and major trials with new therapies have failed to show clinically meaningful benefits.⁴⁻⁶

Serelaxin is a recombinant form of human relaxin-2, a naturally occurring peptide hormone which mediates the physiological cardiovascular (CV) and renal adaptations of pregnancy.^{7,8} In the

RELAX-AHF trial, a 48-h i.v. infusion of serelaxin to patients with AHF was associated with an improvement in dyspnoea, measured by the visual analogue scale (VAS) to Day 5, but without a significant effect on the other primary endpoint of dyspnoea assessed by the Likert scale, and no change in the two secondary endpoints related with 60-day outcomes.⁹ Cardiovascular mortality at Day 180 (a protocol-specified additional efficacy analysis) and all-cause mortality at Day 180 (a pre-specified safety endpoint) were significantly reduced by serelaxin administration, which was consistent with the trend observed in the Pre-RELAX-AHF phase II trial.^{9–11}

Patients hospitalized for HF are a heterogeneous population and differ with respect to many characteristics, including the triggers of

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acute decompensation, and comorbidities.^{1,12–14} The aim of the present study was to compare the effects of serelaxin vs. placebo on dyspnoea, 60-day outcomes and 180-day mortality, in the major subgroups of patients enrolled in the RELAX-AHF trial.

Methods

The background, design and main results of the RELAX-AHF trial (NCT00520806) have been published.^{9,15} Briefly, RELAX-AHF was a phase III randomized, double-blind, placebo-controlled, parallel-group, international trial comparing a 48-h i.v. infusion of serelaxin with placebo in patients hospitalized for AHF. From the start of study drug infusion, patients were followed daily to Day 5 or discharge, and then at Days 14, 60, and, only for mortality, at Day 180.

Study design

Patients eligible for enrolment had to be hospitalized for AHF within 16 h of presentation, with dyspnoea at rest or with minimum exertion, pulmonary congestion on chest radiograph, B-type natriuretic peptide (BNP) \geq 350 ng/L or N-terminal prohormone of BNP (NT-proBNP) \geq 1400 ng/L, mild-to-moderate renal dysfunction [simplified Modification of Diet in Renal Disease estimated glomerular filtration rate (eGFR) of 30–75 mL/min per 1.73 m²], systolic blood pressure (SBP) > 125 mmHg, and treatment with at least 40 mg i.v. furosemide or its equivalent before screening. Exclusion criteria have been described previously.^{9,15}

After randomization, patients received either serelaxin 30 μ g/kg per day or placebo administered as a continuous i.v. infusion for up to 48 h. Protocol-defined dose adjustment rules were applied in case of an excessive SBP decrease or a fall in SBP to below than 100 mmHg or if a serious or intolerable adverse event or clinically significant laboratory abnormality occurred.

The trial had two primary efficacy endpoints: the difference in patientreported dyspnoea as quantified by the area under the curve (AUC) of the change from baseline of dyspnoea severity, reported on a 0 to 100 mm VAS, assessed from baseline to Day 5; and the proportion of patients with a moderate or marked improvement in dyspnoea at 6, 12, and 24 h (all three), assessed by a 7-level Likert scale. Secondary efficacy endpoints were: (i) the rate of the combined endpoint of CV death or rehospitalization for HF or renal failure (RF) to Day 60, and (ii) the days alive and out of the hospital to Day 60 in the serelaxin group, compared with placebo. Cardiovascular deaths to Day 180 and all-cause deaths to Day 180 were assessed as a pre-specified additional efficacy endpoint and a safety endpoint, respectively.

Statistics

An evaluation of the possible interaction between the effect of serelaxin on the two primary and key secondary efficacy endpoints (dyspnoea VAS AUC, moderately or markedly better dyspnoea by Likert, CV death or HF/RF rehospitalization through Day 60, and days alive out of hospital through Day 60) was pre-specified for the following covariates: age (<65 vs. \geq 65 and <75 and \geq 75 years), sex, race (white/Caucasian vs. other), geographic region (Western Europe, Eastern Europe, Israel, USA, Argentina), eGFR (<50 vs. \geq 50 and >60 vs. \geq 66 mL/min/ 1.73 m²), time from presentation to randomization (<6 vs. \geq 6 h), baseline SBP (<140 vs. \geq 140 mmHg), history of diabetes, history of atrial fibrillation, atrial fibrillation present at screening, left ventricular (LV) ejection fraction (LVEF, <40 vs. \geq 40%), history of ischaemic heart disease, history of cardiac resynchronization therapy or implantable cardioverter defibrillator, on i.v. nitrates at randomization. Additional *post hoc* analyses of serelaxin effects on CV and all-cause mortality through Day 180 were also undertaken in these subgroups. Additional subgroups were defined *post hoc* and included hospitalization for HF in the previous year, heart rate (<80 vs. \geq 80 b.p.m.), ACEi/ARB use at baseline, betablocker use at baseline, and lymphocyte proportion (\leq 12 vs. >12%). These covariates were examined as they may modify the effects of AHF therapy and interfere with the vasodilatatory and anti-inflammatory actions of serelaxin.^{7,8} All *P*-values were two-sided, and values <0.05 were considered statistically significant.

Analyses of the efficacy outcomes and CV mortality by baseline levels of biomarkers measured at a central laboratory were also pre-specified: troponin T (>99th percentile of the upper reference limit vs. not), NT-proBNP (by tertiles), and cystatin C (by tertiles). Because few patients had a troponin T value below the 99th percentile of the upper reference limit, this covariate was also grouped by tertiles. As previously described in detail,¹¹ biomarkers were analyzed using the Roche high sensitivity cardiac troponin T assay, the Roche Elecsys proBNP assay and the Gentian Cystatin C immunoassay.

The possible differential effect of serelaxin was tested using a separate regression model for each outcome and each covariate that included the effects of serelaxin, the covariate, and the serelaxin-by-covariate interaction. Treatment effects (odds ratio, mean difference, or hazard ratio) were estimated from these regression models: logistic for moderately/ markedly better dyspnoea at 6, 12, and 24 h; ANCOVA for the VAS AUC to Day 5 and days alive and out of hospital to Day 60; and Cox for the time to CV death or HF/RF rehospitalization to Day 60 and CV and all-cause mortality through Day 180.

Analyses were conducted on an intent-to-treat basis. SAS $^{\odot}$ release 9.2 (SAS Institute, Cary, NC, USA) was used for analysis.

Results

The results of RELAX-AHF have been reported in detail.^{9,11} Enrolment occurred from October 2009 to February 2012 and included 1161 patients (placebo, n = 580; serelaxin, n = 581), of whom 1138 (98%) received randomized study medication. Vital status at 180 days was ascertained for all but 14 patients (two lost-to-follow up; 12 withdrew consent).

Efficacy of serelaxin for subgroups

Patient characteristics, with respect to the baseline variables used to define subgroups, are shown in the *Table 1*.

Subgroup analyses regarding the primary and secondary endpoints of RELAX-AHF and CV and all-cause mortality at Day 180 are shown in *Figures 1–3* and in Supplementary material online, *Figures 1* and 2. The magnitude of the effect of serelaxin relative to placebo on VAS AUC was similar in all the subgroups (*Figure 1*). No differences were also observed by subgroups analysis with respect of the other primary efficacy endpoint of dyspnoea by the Likert scale (Supplementary material online, *Figure 1*) and of the hazard ratio for the secondary endpoint of CV death or HF/RF rehospitalization through Day 60 (*Figure 2, P* > 0.05 in all cases).

Among all the analyses performed on the five clinical endpoints there were five and four nominally significant (P < 0.05) treatmentby-subgroup interactions which were found with the CV and all-cause mortality endpoints, respectively. A larger reduction in CV mortality, with serelaxin vs. placebo, was noted in the patients aged \geq 75 years (P = 0.0337), those with no HF hospitalization in the previous year (P = 0.0119), no beta-blocker use at baseline (P = 0.0432), with blood lymphocytes \leq 12% (P = 0.0137), and with an eGFR

Serelaxin n/N (%)

275/581 (47.3)

306/581 (52.7)

390/581 (67.1)

191/581 (32.9)

387/581 (66.6)

194/581 (33.4)

192/581 (33.0) 389/581 (67.0)

39/581 (6.7)

542/581 (93.3)

110/535 (20.6) 425/535 (79.4)

176/533 (33.0) 182/533 (34.1) 175/533 (32.8)

288/550 (52.4) 262/550 (47.6)

Characteristic		
•••••	Placebo n/N (%)	Serelaxin n/N
Gender		
Male	357/580 (61.6)	368/581 (63.3)
Female	223/580 (38.4)	213/581 (36.7)
Age		
<65 years	119/580 (20.5)	145/581 (25.0)
\geq 65 years	461/580 (79.5)	436/581 (75.0)
<75 years	296/580 (51.0)	315/581 (54.2)
\geq 75 years	284/580 (49.0)	266/581 (45.8)
Region		
Eastern Europe	282/580 (48.6)	280/581 (48.2)
Western Europe	101/580 (17.4)	103/581 (17.7)
South America	37/580 (6.4)	34/581 (5.9)
North America	55/580 (9.5)	59/581 (10.2)
Israel	105/580 (18.1)	105/581 (18.1)
Race		
White/Caucasian	552/580 (95.2)	544/581 (93.6)
Other	28/580 (4.8)	37/581 (6.4)
	· · · · · · · · · · · · · · · · · · ·	
Hospitalization for hear Yes	181/580 (31.2)	214/501 (27 2)
No	399/580 (68.8)	216/581 (37.2) 365/581 (62.8)
110	5777500 (00.0)	505/501 (02.0)
SBP (mmHg)		
<140	284/578 (49.1)	298/577 (51.6)
<u>≥</u> 140	294/578 (50.9)	279/577 (48.4)
Heart rate (b.p.m.)		
<80	296/580 (51.0)	314/581 (54.0)
≥ 80	284/580 (49.0)	267/581 (46.0)
LVEF (%)		
<40	295/539 (54.7)	303/552 (54.9)
≥40	244/539 (45.3)	249/552 (45.1)
History of IHD		
Yes	307/580 (52.9)	296/581 (50.9)
No	273/580 (47.1)	285/581 (49.1)
History of ICD or CRT		452/504 (24 2)
Yes	141/580 (24.3)	153/581 (26.3)
No	439/580 (75.7)	420/381 (/3./)
History of DM		
Yes No	272/580 (46.9) 308/580 (53.1)	279/581 (48.0) 302/581 (52.0)

305/580 (52.6)

275/580 (47.4)

246/579 (42.5)

333/579 (57.5)

Yes

No

Yes

No

AF at screening

272/568 (47.9) 268/564 (47.5) <50 ≥ 50 296/568 (52.1) 296/564 (52.5)

Continued

297/581 (51.1)

284/581 (48.9)

233/580 (40.2)

347/580 (59.8)

MRA use at baseline	
Yes	173/580 (29.8)
No	407/580 (70.2)
IV nitrates at baseline	
Yes	42/580 (7.2)
No	538/580 (92.8)
Lymphocytes at baseline	e
≤12%	127/536 (23.7)
>12%	409/536 (76.3)
Troponin T at baseline ((μg/L)
≤0.024	165/541 (30.5)
0.025-0.045	196/541 (36.2)
>0.045	180/541 (33.3)
NT-proBNP at baseline	(ng/L)
<5000	279/551 (50.6)
≥5000	272/551 (49.4)
≤3346	190/551 (34.5)
3347-7281	174/551 (31.6)

		Lymphocytes at baseline	
•••		≤12%	127/536 (23.7)
		>12%	409/536 (76.3)
		•••••	•••••
		Troponin T at baseline (µ	.g/L)
•••		≤0.024	165/541 (30.5)
		0.025-0.045	196/541 (36.2)
		>0.045	180/541 (33.3)
		•••••	•••••
		NT-proBNP at baseline (ng/L)
		<5000	279/551 (50.6)
		≥5000	272/551 (49.4)
		≤3346	190/551 (34.5)
		3347-7281	174/551 (31.6)
		>7281	187/551 (33.9)

Table | Continued

ACEI/ARB use at baseline

Beta-blocker use at baseline

Time from presentation to randomization

Placebo n/N (%)

266/580 (45.9)

314/580 (54.1)

398/580 (68.6)

182/580 (31.4)

407/580 (70.2)

173/580 (29.8)

Characteristic

<6 h

 \geq 6 h

Yes

No

Yes

No

≤3346	190/551 (34.5)	177/550 (32.2)	
3347-7281	174/551 (31.6)	194/550 (35.3)	
>7281	187/551 (33.9)	179/550 (32.5)	
Cystatin C at baseli	ne (mg/L)		•
≤1.26	180/551 (32.7)	189/550 (34.4)	
1.27-1.65	191/551 (34.7)	180/550 (32.7)	
>1.65	180/551 (32.7)	181/550 (32.9)	
eGFR at baseline (m	nL/min/1.73 m²)		•
<60	408/568 (71.8)	409/564 (72.5)	
≥60	160/568 (28.2)	155/564 (27.5)	
< 50	272/568 (47.9)	268/564 (47 5)	

ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; b.p.m., beats per minute; CRT, cardiac resynchronization therapy; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; h, hours; ICD, implantable cardioverter defibrillator; IHD, ischaemic heart disease; IV, intravenous; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; SBP, systolic blood pressure.

Subgroup		Placebo Mean (SD)	Serelaxin Mean (SD)	Favours placebo	Favours serelaxin	LS mean difference Estimate (95%CI)	Interaction P-value
Total population		2308 (3082)	2756 (2588)			447.7 (120.0, 775.4)	
Gender	Male	2193.7 (3129.0)	2634.5 (2585.3)		⊢ •−	440.8 (26.3, 855.3)	0.9230
	Female	2490.7 (3003.2)	2964.9 (2586.2)		+	474.1 (-60.5, 1008.7)	
Age	<65 years	2325.0 (3210.1)	2634.7 (2582.5)	_	+∙	309.7 (-381.4, 1000.8)	0.6484
	≥65 years	2303.5 (3051.7)	2795.8 (2591.9)			492.3 (119.1, 865.6)	
	<75 years	2483.5 (3042.2)	2675.7 (2482.0)	-	↓●	192.2 (-259.6, 644.0)	0.1113
	≥75 years	2124.9 (3117.8)	2850.3 (2710.5)			725.4 (249.1, 1201.6)	
Region	Eastern Europe	2235.9 (3045.7)	2509.0 (2477.5)		.	273.1 (-196.6, 742.7)	0.8498
	Western Europe	1924.0 (2853.1)	2502.5 (2918.6)			578.6 (-200.9, 1358.1)	
	South America	2771.1 (3441.1)	3674.6 (3058.4)	_		903.5 (-418.9, 2225.9)	
	North America	2428.3 (3337.1)	2877.6 (2751.9)			449.3 (-594.0, 1492.6)	
	Israel		3295.4 (2131.5)			651.1 (-117.2, 1419.3)	
Race	White / Caucasian	2325.2 (3032.0)				375.5 (38.4, 712.6)	0.0960
	Other		3563.2 (3235.4)			►1596.2 (198.6, 2993.7)	
Hosp. for CHF	Yes		2729.2 (2502.3)			488.2 (-74.9, 1051.2)	0.8759
in past year ^P	No		2771.2 (2641.1)			433.0 (28.3, 837.7)	0.0700
SBP						and the second	0.8169
	<140 mmHg		2642.4 (2579.9)			435.8 (-27.2, 898.9)	5.0105
Heart rate ^P	≥140 mmHg		2916.0 (2591.8)			513.4 (46.7, 980.1)	0.6269
near rate	<80 bpm		2837.2 (2550.9)			340.9 (-111.2, 793.0)	0.5358
	≥80 bpm		2659.6 (2633.1)			548.1 (72.4, 1023.8)	
LVEF	<40%		2643.5 (2466.9)		—	378.1 (-71.4, 827.5)	0.8229
	≥40%		2854.7 (2696.9)			454.3 (-40.6, 949.3)	
History of IHD	Yes	2390.5 (3117.9)	2703.9 (2664.0)		+•	313.4 (-141.7, 768.5)	0.4014
	No	2215.0 (3044.3)	2809.3 (2510.8)			594.3 (121.2, 1067.3)	
History of ICD or	Yes	1921.2 (3511.4)	2828.8 (2479.3)			907.6 (256.2, 1558.9)	0.1124
CRT	No	2432.1 (2924.4)	2729.4 (2628.5)		+∙-	297.3 (-81.7, 676.3)	
History of DM	Yes	2285.7 (3216.7)	2998.5 (2583.8)			712.9 (237.5, 1188.2)	0.1279
	No	2327.5 (2963.2)	2531.2 (2576.3)	-	↓ ●−	203.6 (-248.1, 655.4)	
History of AF	Yes	1905.3 (3198.8)	2524.0 (2642.2)			618.7 (166.5, 1070.9)	0.2588
	No	2754.4 (2887.8)	2997.8 (2512.6)	-	.	243.4 (-225.9, 712.7)	
AF at screening	Yes	1959.4 (3135.5)	2500.5 (2592.5)			541.1 (32.0, 1050.3)	0.5910
	No		2926.2 (2578.7)			359.0 (-68.2, 786.3)	•
Time from	<6 h		2797.4 (2586.5)			619.7 (139.4, 1100.0)	0.3398
present. to rand.	≥6 h		2718.1 (2593.5)			299.8 (-148.8, 748.5)	
ACEI/ARB use	Yes	and the state of the second	2645.6 (2585.9)			243.6 (-154.0, 641.2)	0.0763
at baseline ^P	No	2102.2 (3384.4)				878.0 (300.0, 1456.1)	
Beta-blocker	Yes	2271.7 (2988.1)					0.5195
use at baseline ^P						366.0 (-30.4, 762.4)	-
MRA use at	No		2990.9 (2741.8)			597.7 (13.9, 1181.5)	0 1615
baseline ^P	Yes		2511.8 (2314.3)			105.5 (-479.7, 690.6)	0.1615
	No		2875.9 (2708.1)			609.9 (214.1, 1005.7)	0.5500
IV nitrates at baseline	Yes		3017.5 (3597.4)			807.7 (-434.8, 2050.1)	0.5562
	No		2736.8 (2503.6)			421.2 (81.2, 761.2)	0.074
Lymphocytes at baseline ^P	≤12%		2488.4 (3021.2)	-		339.1 (-386.5, 1064.7)	0.8711
	>12%		2812.3 (2485.8)			407.1 (21.2, 793.0)	
Troponin T at baseline ^M	≤0.024 µg/L		3125.9 (2493.8)	-	+	317.8 (-289.2, 924.9)	0.9119
baseline "	0.025–0.045 µg/L	2031.1 (2962.3)	2526.5 (2718.3)		⊢ •−	495.5 (-81.2, 1072.1)	
	>0.045 µg/L	2334.6 (3316.5)	2706.8 (2563.2)		→	372.2 (-222.5, 966.9)	
NT-proBNP at	<5000 ng/L	2443.6 (2990.9)	2781.9 (2529.0)		+	338.3 (-131.6, 808.2)	0.6873
baseline	≥5000 ng/L	2320.8 (3183.3)	2797.6 (2661.4)		—	476.8 (-7.5, 961.0)	
	≤3346 ng/L	2452.3 (2746.5)	2878.5 (2326.2)		+ •-	426.2 (-158.1, 1010.5)	0.3861
	3347-7281 ng/L	2252.6 (3390.6)	2938.5 (2770.3)			686.0 (102.0, 1270.0)	
	>7281 ng/L	2433.8 (3124.8)	2539.4 (2632.9)		•	105.6 (-479.3, 690.5)	10.
Cystatin C at	≤1.26 mg/L	2567.1 (2979.5)	2904.4 (2420.7)	-		337.3 (-244.4, 919.0)	0.9598
baseline	1.27-1.65 mg/L		2501.7 (2920.0)			400.0 (-180.3, 980.2)	
	>1.65 mg/L	. ,	2955.2 (2396.9)			458.0 (-129.9, 1046.0)	
eGFR at	<60 ml/min/1.73m ²	2270.0 (2946.7)	and the second se			503.7 (112.8, 894.6)	0.5537
baseline	≥60 ml/min/1.73m ²		2803.7 (2545.7)	_			5.0001
	<50 ml/min/1.73m ²					279.9 (-349.7, 909.5)	0.8254
		2002.1 (0000.0)	2754.0 (2580.3)			401.3 (-79.7, 882.2)	0.0204

-2500-2000-1500-1000-500 0 500 1000 1500 2000 2500 Least squares mean difference in dyspnoea (VAS AUC) to Day 5

Figure I Forest plots of subgroup analysis for dyspnoea Visual Analogue Scale area under the curve change from baseline to Day 5. Post hoc subgroup analysis, ^MModification of categories for pre-specified subgrouping variable. ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; b.p.m., beats per minute; CI, confidence interval; CRT, cardiac resynchronization therapy; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; h, hours; hosp., hospitalization; ICD, implantable cardioverter defibrillator; IHD, ischaemic heart disease; IV, intravenous; LS, least squares; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; MRA, mineralocorticoid receptor antagonist; present., presentation; rand., randomization; SBP, systolic blood pressure; SD, standard deviation.

Subgroup		Placebo n/N (K-M%)	Serelaxin n/N (K-M%)	Favours serelaxin	Favours placebo	Hazard ratio Estimate (95%CI)	Interaction P-value
Total population		75/580 (13.0)	76/581 (13.2)		-	1.02 (0.74, 1.41)	0 7050
Gender	Male	47/357 (13.3)	47/368 (12.9)			0.98 (0.65, 1.46)	0.7250
Age	Female	28/223 (12.6)	29/213 (13.7)			1.10 (0.65, 1.85)	0.9880
Age	<65 years ≥65 years	14/119 (11.8) 61/461 (13.4)	17/145 (12.0)				0.9880
	<75 years	35/296 (11.8)	59/436 (13.6) 38/315 (13.2)			1.03 (0.72, 1.47) 1.04 (0.66, 1.64)	0.9474
	≥75 years	40/284 (14.3)	38/315 (12.2) 38/266 (14.4)		_	1.02 (0.65, 1.58)	0.9474
Region	Eastern Europe	32/282 (11.4)	29/280 (14.4)			0.92 (0.56, 1.58)	0.9346
Region	Western Europe	14/101 (14.3)	13/103 (13.1)			0.92 (0.38, 1.33)	0.9346
	South America	4/37 (10.8)	6/34 (17.7)		_	1.61 (0.45, 5.71)	-
	North America	9/55 (16.4)	11/59 (18.6)			1.15 (0.48, 2.78)	-
	Israel	16/105 (15.2)	17/105 (16.3)			1.08 (0.55, 2.14)	-
Race	White/Caucasian	69/552 (12.6)	71/544 (13.2)			1.06 (0.76, 1.47)	0.3688
Nace	Other	6/28 (21.4)	5/37 (13.5)			0.60 (0.18, 1.97)	- 0.5000
Hosp. for CHF in	Yes		46/216 (21.7)		_	and the second se	0.1506
past year ^P	No	33/181 (18.3) 42/399 (10.6)			_	1.24 (0.79, 1.93)	0.1506
SBP			30/365 (8.3)			0.77 (0.48, 1.23)	0.4290
001	<140 mmHg	43/284 (15.3)	49/298 (16.7)		_	1.11 (0.74, 1.67)	0.4389
Heart rate ^P	≥140 mmHg	32/294 (11.0)	26/279 (9.4)		-	0.85 (0.51, 1.43)	0.2162
nealt late.	<80 b.p.m	34/296 (11.5)	44/314 (14.1)			1.25 (0.80, 1.95)	0.2102
LVEF	≥80 b.p.m	41/284 (14.6) 38/295 (13.0)	32/267 (12.2)			0.83 (0.52, 1.32)	0.4075
	<40% ≥40%	38/295 (13.0) 30/244 (12.4)	37/303 (12.3) 38/249 (15.4)		_	0.96 (0.61, 1.51)	0.4075
History of IHD	Yes	47/307 (15.5)	CONTRACTOR AND A DESCRIPTION OF A DESCRI		-	1.27 (0.78, 2.04)	0.0000
history of IHD	No		44/296 (15.0)		_	0.98 (0.65, 1.47)	0.6983
History of ICD or		28/273 (10.3)	32/285 (11.4)		—	1.11 (0.67, 1.85)	0.1666
History of ICD or CRT	Yes	31/141 (22.3)	25/153 (16.6)		-	0.75 (0.44, 1.27)	0.1666
History of DM		44/439 (10.1)	51/428 (12.0)		-	1.20 (0.80, 1.79)	0.8324
HISTOLY OF DIVI	Yes	43/272 (15.9)	45/279 (16.3)		—	1.05 (0.69, 1.60)	0.8324
History of AF	No	32/308 (10.5)	31/302 (10.4)			0.98 (0.60, 1.61)	0.6488
HISTOLY OF AF	Yes	47/305 (15.5)	44/297 (15.0)		_	0.97 (0.64, 1.46)	0.0400
AE at assaults		28/275 (10.3)	32/284 (11.4)		-	1.12 (0.68, 1.87)	0.4004
AF at screening	Yes	39/246 (16.0)	30/233 (13.0)			0.79 (0.49, 1.28)	0.1801
Time from	No	36/333 (10.9)	45/347 (13.1)		•—	1.24 (0.80, 1.92)	0.0500
Time from present. to rand.	<6 h	40/266 (15.1)	39/275 (14.4)		_	0.95 (0.61, 1.48)	0.6563
ACEI/ARB use at	≥6 h	35/314 (11.3)	37/306 (12.2)		-	1.10 (0.69, 1.74)	0.0057
baseline ^P		42/398 (10.6)	51/390 (13.2)		-	1.27 (0.84, 1.91)	0.0857
	No	33/182 (18.3)	25/191 (13.2)		-	0.71 (0.42, 1.19)	0.0000
Beta-blocker use at baseline ^P	Yes	46/407 (11.4)	49/387 (12.8)		•—	1.14 (0.76, 1.71)	0.3239
MRA use at	No	29/173 (16.8)	27/194 (14.1)		_	0.82 (0.49, 1.38)	0.4000
baseline ^P	Yes	21/173 (12.2)	32/192 (17.0)			1.44 (0.83, 2.50)	0.1239
IV nitrates at	No	54/407 (13.4)	44/389 (11.4)			0.85 (0.57, 1.26)	0.0757
baseline	Yes	8/42 (19.5)	5/39 (12.8)			0.63 (0.21, 1.93)	0.3757
	No (12%)	67/538 (12.5)	71/542 (13.3)		-	1.07 (0.77, 1.49)	0 1200
Lymphocytes at baseline ^P	≤12% >12%	27/127 (21.6)	15/110 (13.8)			0.61 (0.32, 1.14)	0.1260
Troponin T at	>12%	44/409 (10.8)	49/425 (11.6)			1.09 (0.73, 1.64)	0.7500
baseline ^M	≤0.024 µg/L	17/165 (10.4)	15/176 (8.5)		_	0.81 (0.41, 1.63)	0.7528
States - Second - C	0.025–0.045 µg/L >0.045 µg/L	23/196 (11.8) 30/180 (16.8)	24/182 (13.2)			1.15 (0.65, 2.03)	-
	10	. ,	30/175 (17.5)		_	1.04 (0.63, 1.73)	0.0107
NT-proBNP at baseline	<5000 ng/L	29/279 (10.4)	32/288 (11.2)			1.08 (0.65, 1.79)	0.9127
	≥5000 ng/L	42/272 (15.6)	42/262 (16.1)			0.58 (0.27, 1.26)	0.1629
	≤3346 ng/L	18/190 (9.5)	10/177 (5.7)		-		0.1628
	3347-7281 ng/L	22/174 (12.7)	34/194 (17.8)	1		1.45 (0.85, 2.47)	-
Cystatin C at	>7281 ng/L	31/187 (16.8) 13/180 (7.3)	30/179 (16.8) 16/189 (8.6)		_	1.01 (0.61, 1.68)	0.8000
baseline	≤1.26 mg/L		, ,			1.19 (0.57, 2.47)	0.8900
	1.27–1.65 mg/L	22/191 (11.7)	20/180 (11.2)			0.95 (0.52, 1.74)	-
	>1.65 mg/L	36/180 (20.1)	38/181 (21.1)		-	1.08 (0.69, 1.71)	0.0440
	<60 mL/min/1.73m ²		53/409 (13.1)	-•	-	0.90 (0.62, 1.30)	0.3113
	≥60 mL/min/1.73m ²		18/155 (11.7)			1.35 (0.67, 2.72)	0.4015
	<50 mL/min/1.73m ² ≥50 mL/min/1.73m ²		40/268 (15.2)	-•	_	0.89 (0.58, 1.35)	0.4245
			31/296 (10.5)			1.16 (0.69, 1.95)	

Figure 2 Forest plots of subgroup analysis for cardiovascular death or heart failure or renal failure rehospitalization through Day 60. ^P*Post hoc* subgroup analysis, ^MModification of categories for pre-specified subgrouping variable. ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; b.p.m., beats per minute; CI, confidence interval; CRT, cardiac resynchronization therapy; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; h, hours; hosp., hospitalization; ICD, implantable cardioverter defibrillator; IHD, ischaemic heart disease; IV, intravenous; K–M, Kaplan–Meier; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; MRA, mineralocorticoid receptor antagonist; present., presentation; rand., randomization; SBP, systolic blood pressure.

Subgroup		Placebo n/N (K–M%)	Serelaxin n/N (K–M%)	Favours serelaxin	Favours placebo	Hazard ratio Estimate (95%CI)	Interaction P-value
Total population Gender		54/580 (9.4)	34/581 (5.9)			0.62 (0.40, 0.95)	0.0047
Gender	Male	32/357 (9.1)	25/368 (6.9)		-	0.75 (0.44, 1.26)	0.2317
A.g.o.	Female	22/223 (10.0)	9/213 (4.3)			0.42 (0.19, 0.92)	0.5022
Age	<65 years	9/119 (7.6)	9/145 (6.3)			0.82 (0.33, 2.07)	0.5023
	≥65 years	45/461 (9.9)	25/436 (5.8)			0.58 (0.35, 0.94)	0.0337
	<75 years	18/296 (6.1)	20/315 (6.5)			1.05 (0.55, 1.98)	0.0337
Decise	≥75 years	36/284 (12.9)	14/266 (5.3)			0.40 (0.22, 0.74)	0.7540
Region	Eastern Europe	32/282 (11.5)	22/280 (7.9)		F	0.68 (0.40, 1.18)	0.7540
	Western Europe	7/101 (7.1)	2/103 (2.0)	• •	—	0.27 (0.06, 1.30)	-
	South America	4/37 (10.9)	3/34 (8.8)	•		0.82 (0.18, 3.65)	-
	North America	4/55 (7.3)	4/59 (6.9)			0.91 (0.23, 3.64)	
_	Israel	7/105 (6.8)	3/105 (2.9)			0.43 (0.11, 1.66)	
Race	White/Caucasian	51/552 (9.4)	32/544 (6.0)			0.63 (0.40, 0.98)	0.7573
	Other	3/28 (10.7)	2/37 (5.5)	← →		0.47 (0.08, 2.81)	
Hosp. for CHF in	Yes	13/181 (7.2)	19/216 (9.0)		• -	1.26 (0.62, 2.54)	0.0119
past year	No	41/399 (10.4)	15/365 (4.1)	— •—		0.39 (0.21, 0.70)	
SBP	<140 mmHg	30/284 (10.8)	21/298 (7.2)		-	0.65 (0.37, 1.14)	0.7403
	≥140 mmHg	24/294 (8.2)	13/279 (4.7)		-	0.56 (0.29, 1.11)	
Heart rate	<80 bpm	23/296 (7.8)	16/314 (5.1)		-	0.65 (0.34, 1.23)	0.8777
	≥80 bpm	31/284 (11.1)	18/267 (6.9)			0.61 (0.34, 1.08)	
LVEF	<40%	24/295 (8.2)	21/303 (7.0)		_	0.85 (0.47, 1.52)	0.1410
	≥40%	25/244 (10.4)	11/249 (4.5)			0.42 (0.21, 0.86)	_
History of IHD	Yes	31/307 (10.3)	17/296 (5.8)			0.55 (0.31, 1.00)	0.5789
	No	23/273 (8.5)	17/285 (6.1)		_	0.71 (0.38, 1.32)	
History of ICD or	Yes	15/141 (10.8)	15/153 (10.0)			0.92 (0.45, 1.88)	0.1691
CRT	No	39/439 (9.0)	19/428 (4.5)			0.49 (0.28, 0.85)	-
History of DM	Yes	27/272 (10.0)	16/279 (5.8)			0.57 (0.31, 1.06)	0.7277
,	No	27/308 (8.9)	18/302 (6.1)		L	0.67 (0.37, 1.21)	-
History of AF	Yes	32/305 (10.7)	20/297 (6.8)		F	0.63 (0.36, 1.10)	0.9486
notory or ru	No	22/275 (8.1)	14/284 (5.0)			0.61 (0.31, 1.19)	-
AF at screening	10.070				F		0.1410
Ar at screening	Yes	30/246 (12.4)	13/233 (5.6)			0.44 (0.23, 0.84)	- 0.1410
Time from	No	24/333 (7.3)	21/347 (6.2)		_	0.84 (0.47, 1.52)	0.4570
Time from present. to rand.	<6 h	23/266 (8.7)	20/275 (7.4)			0.84 (0.46, 1.53)	0.1576
	≥6 h	31/314 (10.0)	14/306 (4.6)			0.45 (0.24, 0.85)	0.4000
ACEI/ARB use at baseline	100	32/398 (8.1)	24/390 (6.2)		–	0.76 (0.45, 1.30)	0.1920
	No	22/182 (12.3)	10/191 (5.3)			0.41 (0.20, 0.88)	
Beta-blocker use at baseline	Yes	32/407 (8.0)	26/387 (6.8)	•	-	0.85 (0.50, 1.42)	0.0432
	No	22/173 (12.7)	8/194 (4.2)			0.31 (0.14, 0.71)	
MRA use at baseline	Yes	18/173 (10.5)	16/192 (8.5)		-	0.81 (0.41, 1.59)	0.2954
	No	36/407 (9.0)	18/389 (4.7)			0.51 (0.29, 0.89)	
IV nitrates at	Yes	7/42 (17.0)	3/39 (7.8)		_	0.44 (0.11, 1.70)	0.5987
baseline	No	47/538 (8.8)	31/542 (5.8)			0.65 (0.41, 1.02)	
Lymphocytes at baseline	≤12%	25/127 (20.2)	6/110 (5.6)	— •—		0.25 (0.10, 0.62)	0.0137
Jaseime	>12%	28/409 (6.9)	27/425 (6.4)		_	0.93 (0.55, 1.58)	
Troponin T at	≤0.024 µg/L	6/165 (3.7)	5/176 (2.8)	+		0.78 (0.24, 2.55)	0.1929
baseline	0.025–0.045 µg/L	11/196 (5.7)	11/182 (6.1)		•	1.08 (0.47, 2.50)	
	>0.045 µg/L	32/180 (18.0)	14/175 (8.2)			0.42 (0.23, 0.79)	-
NT-proBNP at	<5000 ng/L	12/279 (4.3)	12/288 (4.2)		-	0.97 (0.44, 2.16)	0.2761
oaseline	≥5000 ng/L	37/272 (13.9)				0.57 (0.33, 0.97)	
	≤3346 ng/L	5/190 (2.7)	4/177 (2.3)			0.85 (0.23, 3.17)	0.8700
	3347-7281 ng/L	17/174 (9.9)	13/194 (6.8)		_	0.69 (0.33, 1.42)	
	>7281 ng/L	27/187 (14.8)	16/179 (9.0)			0.59 (0.32, 1.10)	-
Cystatin C at	≤1.26 mg/L	5/180 (2.8)	10/189 (5.4)	_		1.93 (0.66, 5.63)	0.0739
paseline	1.27–1.65 mg/L	18/191 (9.7)	7/180 (3.9)		-	0.40 (0.17, 0.95)	
	>1.65 mg/L	26/180 (14.6)	16/181 (8.9)		L	0.60 (0.32, 1.11)	
eGFR at baseline	<60 mL/min/1.73m ²	46/408 (11.5)	25/409 (6.2)			0.53 (0.33, 0.86)	0.1056
	≥60 mL/min/1.73m ²	6/160 (3.8)	8/155 (5.2)			1.39 (0.48, 4.00)	-
	And the second se						0.0319
	<50 mL/min/1.73m ²	37/272 (13.9)				0.42 (0.23, 0.76)	0.0019
	≥50 mL/min/1.73m ²	15/296 (5.1)	17/296 (5.8)		-	1.14 (0.57, 2.29)	

Figure 3 Forest plots of subgroup analysis for cardiovascular death through Day 180. All these analyses were *post hoc*. ACEI, angiotensinconverting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; b.p.m., beats per minute; CI, confidence interval; CRT, cardiac resynchronization therapy; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; h, hours; hosp., hospitalization; ICD, implantable cardioverter defibrillator; IHD, ischaemic heart disease; IV, intravenous; K-M, Kaplan-Meier; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; MRA, mineralocorticoid receptor antagonist; present., presentation; rand., randomization; SBP, systolic blood pressure. <50 mL/min/m² (P = 0.0319) (Figure 3). Similar data were found when 180-day all-cause mortality was considered. The reduction in all-cause mortality, with serelaxin vs. placebo, was larger in the patients aged \geq 75 years (P = 0.0473), with no HF hospitalization in the previous year (P = 0.0222), with blood lymphocytes \leq 12% (P = 0.0298) and with an eGFR <50 mL/min/m² (P = 0.0286). No significant interaction was found with any other covariate (Supplementary material online, Figure 2).

Discussion

Our study shows consistency of the results of the RELAX-AHF trial across different patient subgroups based on baseline clinical characteristics. These subgroups included all the pre-specified subgroups as well as additional ones generally considered as clinically important in AHF studies.

Patients hospitalized for HF are known to be a heterogeneous group of patients. The main pathophysiological mechanisms leading to HF decompensation may range from severe LV systolic dysfunction with low cardiac output, low blood pressure, peripheral hypoperfusion, fluid retention and oedema, to increased vascular stiffness, increased pre- and afterload, impairment of LV diastolic function, pulmonary congestion, and oedema.^{1,13,14} RELAX-AHF was among the first trials to take these differences into account and specifically target those patients most likely to benefit from the study drug.¹⁵

Our present analysis shows that the effects of serelaxin on study outcomes were generally consistent across subgroups. Specifically, there was no interaction between the effects of serelaxin and critical variables (such as SBP, time from presentation to randomization, and baseline NT-proBNP values). However, since the study was designed to include patients with SBP > 125 mmHg, within 16 h from presentation and with relatively high baseline NT-proBNP or BNP levels, it cannot be excluded that the patients selected were sufficiently homogenous so that subgroup analysis could not yield any further selection of the patients with a better response. Thus, while generally representative of most of the AHF patients, inclusion/exclusion criteria of RELAX-AHF may have resulted in the selection of a relatively homogeneous population, more likely to benefit from a treatment with a predominant vascular mode of action, and with no evident subgroup differences. Secondly, the drug may act on some mechanisms which contribute to the symptoms and the poor survival of the patients having characteristics similar to those of the patients enrolled in RELAX-AHF.^{7,8}

Systolic blood pressure is a major prognostic factor in patients hospitalized for AHF.^{1,12} It is also a major determinant of the effects of therapy, with the untoward effects of new therapies associated with an excessive blood pressure drop.^{6,16,17} Based on these data, a SBP >125 mmHg was selected as entry criterion for the RELAX-AHF trial.^{9,15} This criterion still allows the inclusion of > 70% of the patients admitted for AHF.^{1,12} Despite its vasodilatatory effects, also shown by the larger proportion of patients who had had a protocol-defined blood-pressure-related study drug dose adjustment or study drug discontinuation,⁹ no interaction was found between the effects of serelaxin, compared with placebo, and the baseline SBP.

Early treatment is considered essential to obtain better effects in patients admitted for AHF. In analyses of patients included in the Acute Decompensated Heart Failure (ADHERE) Registry, Peacock et al.¹⁸ showed that early administration of vasodilators was associated with a better outcome, compared with late treatment. In the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial, patients could be enrolled 24 h from the start of furosemide treatment and the median time from the start of treatment to randomization was 16 h.¹⁹ However, these considerations were already taken into account in the design of RELAX-AHF, where patients were enrolled within 16 h from presentation with the median time from presentation to randomization being 7.9 ± 4.7 and 7.8 ± 4.6 h, in the placebo and the serelaxin groups, respectively.9 It is possible that the relatively short time from presentation may have reduced the interpatient variability and, therefore, the interaction between time from presentation to randomization and serelaxin's effect.

N-terminal prohormone of BNP is a major prognostic variable in most studies of patients hospitalized for HF.^{4,20,21} Increased NT-proBNP or BNP levels at baseline were required as inclusion criteria in RELAX-AHF, with the aim to avoid the enrolment of patients without congestion. This criterion has allowed the enrolment of patients at relatively high risk of events. However, also in this case, baseline NT-proBNP levels did not influence the effects of serelaxin vs. placebo and this may be related to the pre-selection of patients with high BNP or NT-proBNP levels at baseline, since this was an inclusion criterion.¹¹

Patients with a preserved LVEF represent approximately half of the patients admitted for AHF and this proportion is growing due to ageing of the general population.²² No treatment has been shown as effective in the patients with HF and preserved LVEF, to date.⁴ These patients have unique clinical characteristics and were excluded or under-represented in previous large AHF trials.^{16,23,24} Less than 20% of all the patients included in ASCEND-HF had a LVEF <40%,¹⁹ and LVEF was available in only approximately half of all the patients in two other AHF trials.^{25,26}

In RELAX-AHF, LVEF was available in most of the patients (*Table 1*) and 45% of the patients had a LVEF above the pre-specified cut-off for subgroup analysis of 40%.⁹ No differences in the response to sere-laxin administration were found in the patients with a LVEF < or \geq 40% with respect to any endpoint. These results are consistent with the presence of common pathogenetic mechanisms in AHF, such as increased LV afterload, pulmonary congestion, and end-organ damage, which are independent of LVEF, and on which serelaxin may exert beneficial effects.^{11,27} These results are also consistent with serelaxin's mechanism of action, which is active more on the peripheral vasculature and arterial elastance, rather than on the myocardium.^{7,8}

Another subgroup of potential interest is that of patients on i.v. nitrates at the time of randomization. Nitrates are a treatment option for patients with AHF and high blood pressure⁴ and were allowed in RELAX-AHF for the patients with a SBP >150 mmHg at screening.^{9,15} Although, theoretically, their concomitant administration might have blunted some of the favourable effects of serelaxin, estimated treatment effects on mortality and dyspnoea were in the same direction as for the patients not on nitrates. However, the

proportion of patients on i.v. nitrates at randomization was small and the confidence intervals for the estimated treatment effects were wide.

Some interactions were nominally statistically significant when the 180-day CV mortality and all-cause mortality data were considered (Figure 3). However, these data have to be interpreted with caution as these subgroup analyses were not pre-specified, the number of subgroups examined was large and the number of events was small. The interaction between some variables and the effects of serelaxin on mortality, compared with placebo, may be explained by the low incidence of deaths in the subgroups at lower risk, such as that of the patients \leq 75 years old or with an eGFR \geq 50 mL/min/m². Some data seem, however, to suggest that serelaxin was more effective in patients with no history of HF, as shown by the subgroups with no HF hospitalization in the previous year and no concomitant treatment with neurohormonal antagonists, such as angiotensinconverting enzyme inhibitors, mineralocorticoid receptor antagonists and beta-blockers (Figures 1-3). One potential explanation is that patients not treated with neurohormonal antagonists are more likely to be more sensitive to the untoward effects of diuretic therapy, and serelaxin administration was associated with lower diuretic doses in the RELAX-AHF trial.⁹ The same might also apply to the subgroup with reduced eGFR as kidney dysfunction is also associated with the need to use higher doses of loop diuretics. In addition, serelaxin may be effective on neurohormonal and inflammatory mechanisms which are favourably affected also by neurohormonal antagonists.7,8

The limitations of subgroup analyses are well known.^{28,29} Although the results suggest a homogeneous response to serelaxin among the subgroups examined, the study was not powered to detect differential treatment effects. On the other hand, any nominally statistically significant interactions should be interpreted cautiously given the number of both pre-specified and *post hoc* analyses performed. The results of the present study must be limited to the patients with characteristics similar to those of the patients enrolled in RELAX-AHF with, namely, a SBP >125 mmHg and <16 h from presentation to hospital. Any extension of the present findings to patients with different characteristics from those of the patients in RELAX-AHF is not possible.

In conclusion, subgroup analyses of the RELAX-AHF trial has shown similar effects of serelaxin, when compared with placebo, across various subgroups, suggesting a consistency of the effect of serelaxin in the patients with AHF with the characteristics used in this study.

Supplementary material

Supplementary material is available at European Heart Journal online.

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