ESC European Society of Cardiology

European Journal of Heart Failure (2025)
doi:10.1002/ejhf.3764

RESEARCH ARTICLE

Coordinated expression of BMP10/ALK1/endoglin—proteins that drive embryonic cardiac and vascular morphogenesis—in patients with heart failure: The EMPEROR Program

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Received 17 April 2025; revised 18 June 2025; accepted 26 June 2025

Aims

Bone morphogenetic protein 10 (BMP10), activin receptor-like kinase 1 (ALK1) and endoglin form a single transforming growth factor-β family signalling complex that is the principal driver of cardiac and vascular morphogenesis and maturation during hypoxic embryonic development. These proteins are down-regulated with the onset of normoxia at birth, but are up-regulated following experimental cardiac injury. Yet, little is known about the expression of this protein complex in patients with heart failure.

Methods and results

In the EMPEROR Program, we measured serum levels of BMP10 by electrochemiluminescence immunoassay in 1127 patients in Cohort 1 ($n = 1127$) and plasma levels of BMP10, ALK1 and endoglin by proximity extension assay in a distinct Cohort 2 ($n = 1120$). In both cohorts, patients were characterized at baseline and were followed for the occurrence of major adverse heart failure events. Levels of BMP10, ALK1 and endoglin at baseline and changes in these levels during follow-up were closely correlated with each other. Higher levels of BMP10, ALK1 and endoglin were associated with worse functional class, higher likelihood of atrial fibrillation and higher levels of natriuretic peptides and troponin T (p for trend <0.001 for all). Increasing levels of BMP10, ALK1 and endoglin were associated with progressively higher risks of major adverse outcomes (p for trend <0.001 for all three proteins and for all heart failure endpoints). The hazard ratios for the risks associated with tertiles of the three proteins in Cohort 2 were remarkably similar to those seen with BMP10 in Cohort 1. Treatment with empagliflozin had a modest effect to reduce BMP10 in both cohorts.

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Conclusions The coordinated circulating expression of proteins critical to foetal cardiac and vascular development tracks closely with the severity of heart failure, as reflected by symptoms, cardiac injury and stress, prevalence of atrial fibrillation and other comorbidities, and prognosis, suggesting a role of BMP10/ALK1/endoglin signalling in the progression of heart failure.

Keywords Expression of foetal genes • Heart failure • BMP10 • ALK1 • Endoglin

Introduction

When the adult heart is injured or stressed, it recapitulates the foetal gene programming that played an essential role in embryonic cardiac morphogenesis.[1–3] Anabolic signalling pathways that thrived under hypoxic low-workload conditions are reactivated, but such up-regulation—occurring under conditions of normoxia and adult levels of systemic blood pressure—can lead to maladaptive hypertrophy and cardiac fibrosis.[2,4]

Foetal reprogramming-related up-regulation of hypoxia-inducible factor-1 alpha (HIF-1α) in heart failure is particularly noteworthy.[5,6] It is responsible for the shift of the failing heart to heightened glucose uptake and glycolysis,[7] a pattern that recapitulates embryonic cardiac development.[8] Additionally, embryonic up-regulation of HIF-1α expression plays a critical role in cardiomyocyte differentiation and cardiac morphogenesis.[9–11] HIF-1α signalling is suppressed with the onset of normoxia at birth, but re-expression of HIF-1α in the adult heart following injury promotes cardiac fibrosis, which is mediated through its downstream action on members of the transforming growth factor-β (TGF-β) superfamily.[12,13] Up-regulation of HIF-1α/TGF-β plays a well-defined role in both atrial and ventricular fibrosis in hearts under stress.[6,12–15]

A key member of the TGF-β superfamily, bone morphogenetic protein 10 (BMP10) plays an essential role in the heart. HIF-1α-mediated heightened glucose uptake promotes the expression of BMP10 during cardiac embryogenesis.[16,17] In the foetal heart, BMP10 is a principal driver of cardiac morphogenesis, being particularly enriched in trabecular myocardium.[18–20] Loss-of-function polymorphisms in the BMP10 gene are linked to congenital heart defects and dilated cardiomyopathy.[21–23] BMP10 signals through the activin receptor-like kinase 1 (ALK1) (also known as activin type I receptor) to promote vascular development and maintenance,[24–26] and ALK1 is essential for embryonic angiogenesis and maturation.[26,27] Importantly, BMP10 expression is suppressed following birth,[28] when normoxia leads to the cessation of cardiac hyperplastic responses, and BMP10 becomes restricted to the right atrium in post-natal hearts.[29,30] Similarly, following birth, low expression levels of ALK1 promote vascular quiescence,[28,31] and human loss-of-function ALK1 mutations lead to arteriovenous malformations in hereditary haemorrhagic telangiectasia.[32]

The role of BMP10 and ALK1 in modulating the responses to cardiac stress and injury in the adult heart is not well-defined. Experimentally-induced overexpression of BMP10 exerts cardioprotective effects following catecholamine stimulation, doxorubicin exposure and myocardial infarction,[33–35] and experimental knockout of ALK1 impairs adaptive hypertrophic responses, promotes cardiac fibrosis and leads to heart failure.[36,37] However, these extreme conditions do not inform an understanding of the measured responses of BMP10 and ALK1 to cardiac injury and stress in adulthood. It is therefore noteworthy that BMP10 is up-regulated in the myocardium during stress[29,30] and may mediate maladaptive cardiac hypertrophy,[30,38] and enhanced ALK1 signalling has been implicated in the promotion of pro-fibrotic pathways.[39,40] In clinical studies, heightened circulating BMP10 levels have been associated with left atrial endomysial fibrosis and atrial fibrillation in patients undergoing cardiac surgery,[41] and they predict worsening heart failure events in patients with atrial fibrillation.[42,43] One paper reported that circulating BMP10 levels were correlated with both atrial fibrillation and atrial size in patients with heart failure, suggesting that the protein was an indicator of atrial stress.[44]

Signalling of BMP10 through ALK1 is modulated by circulating levels of soluble endoglin, a glycoprotein co-receptor.[45] During embryonic development, endoglin promotes epithelial-to-mesenchymal transition, leading to the development of the endocardium and valve structures and the maturation of endothelial cells in the vasculature.[46–48] As with BMP10 and ALK1, endoglin is typically expressed at low levels in post-natal endothelial cells.[49] However, in states of adult cardiac injury, endoglin is up-regulated in cardiac fibroblasts,[50] and the formation of BMP10/ALK1/endoglin complex triggers a molecular switch that leads TGF-β family members (including BMP10) to activate pro-inflammatory and pro-fibrotic pathways.[49,51–54] Soluble endoglin is increased following mechanically-provoked cardiac injury,[55] and experimental suppression of endoglin limits cardiac fibrosis and enhances survival in heart failure.[56,57] Heightened levels of soluble endoglin identify patients with coronary artery disease at risk of major cardiovascular events[50] and patients with suspected left ventricular dysfunction who have elevated left ventricular filling pressures and greater functional impairment.[58] Endoglin expression is increased in the left ventricles of patients with advanced heart failure and is down-regulated following left ventricular assist device implantation.[56]

Despite the biological role of BMP10/ALK1/endoglin in the heart, little is known about the coordinated expression of this pathway in patients with heart failure or the effects of pharmacological treatment. Accordingly, in the EMPEROR-Reduced (Empagliflozin Outcome Trial in Patients with Chronic Heart Failure and a Reduced Ejection Fraction) and EMPEROR-Preserved (Empagliflozin Outcome Trial in Patients with Chronic Heart Failure with Preserved Ejection Fraction) trials,[59,60] we measured serum levels of BMP10 before and after treatment with placebo or the sodium–glucose co-transporter 2 (SGLT2) inhibitor

empagliflozin using electrochemiluminescence immunoassay in one cohort, and then, we measured BMP10/ALK1/endoglin by proximity extension assay in a distinct and separate cohort. In doing so, we determined (i) if circulating levels of BMP10, ALK1 and endoglin are highly aligned with each other and track closely with the severity of heart failure; (ii) if the observed clinical associations of circulating BMP10 are assay-independent; (iii) if SGLT2 inhibition influences circulating levels of BMP10, ALK1 and endoglin during short- and long-term treatment; and (iv) if circulating levels of BMP10, ALK1 and endoglin influence the response to SGLT2 inhibition.

Methods

The EMPEROR Program consisted of two phase III, international, multicentre, double-blind, randomized, parallel-group, placebo-controlled trials—the EMPEROR-Reduced and the EMPEROR-Preserved trials. The trials were run concordantly using similar study protocols, administrative structures and study sites, and similar study monitoring and statistical analysis plans. The primary difference between the two trials was the inclusion of patients with a left ventricular ejection fraction ≤40% in the EMPEROR-Reduced and >40% in the EMPEROR-Preserved trial.[59,60]

Patient population

Participants were men or women who had chronic heart failure and elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, i.e. >300 pg/ml if the ejection fraction was >40%; ≥2500 pg/ml if the ejection fraction was 36–40%; ≥1000 pg/ml if the ejection fraction was 31–35%; and ≥600 pg/ml if the ejection fraction was ≤30% or if they had been hospitalized for heart failure within the prior 12 months. If patients had atrial fibrillation, the thresholds for NT-proBNP were doubled in EMPEROR-Reduced and tripled in EMPEROR-Preserved. Patients were randomized to receive either placebo or empagliflozin 10 mg daily, which was maintained for the duration of double-blind therapy (median follow-up of 16 months in EMPEROR-Reduced and 26 months in EMPEROR-Preserved). The protocol was approved by the Ethics Committee of all participating sites, and all patients provided written informed consent before enrolment. A separate consent was obtained for blood sampling and biobanking to allow for the measurement of circulating proteins and other biochemical assessment.

Biomarker measurements

Of the 9718 patients enrolled in the EMPEROR Program, 5030 patients agreed to blood biobanking, and among these, two distinct cohorts of patients were randomly identified. In Cohort 1 (consisting of 1127 patients), we measured BMP10 by an electrochemiluminescence immunoassay (Roche Diagnostics) in serum samples collected at baseline and after 12 and 52 weeks. Baseline high-sensitivity cardiac troponin T (hs-cTnT) and NT-proBNP were also measured (using the Roche Cobas® platform) at these time points. These assays provide absolute concentrations in the blood samples. In Cohort 2 (consisting of 1120 patients), we measured BMP10, ALK1 and endoglin at baseline and at 12 and 52 weeks in plasma samples using the Olink® Explore 3072 platform, utilizing proximity extension assay technology with a dual-recognition DNA-coupled readout.[9] The platform provides $\log_2$ normalized protein expression values with relative quantification, but it does not provide absolute concentrations. No patient was included in both cohorts.

Trial outcomes and statistical analyses

The primary endpoint for both trials was the composite of cardiovascular death or first hospitalization for heart failure. We also analysed the individual components of the primary endpoint, all-cause mortality, and total hospitalizations for heart failure.

Study participants were categorized according to the tertiles of baseline BMP10 concentrations or expression levels (for Cohort 1 and Cohort 2, respectively), and in Cohort 2, study participants were also categorized according to the tertiles of baseline ALK1 and endoglin levels. Baseline characteristics of the patients across the tertiles of BMP10, ALK1 and endoglin were compared using ordinal regression. For both cohorts, the analyses of the associations between BMP10 concentration (Cohort 1) and BMP10, ALK1 and endoglin expression levels (Cohort 2) with the time-to-event endpoints were performed using a Cox regression model; for the analyses of total heart failure hospitalizations, a joint frailty model was used with cardiovascular death as a competing risk. For all endpoints, we adjusted for the protocol pre-specified covariates of age, estimated glomerular filtration rate, left ventricular ejection fraction, region, diabetes, sex, treatment (empagliflozin or placebo), as well as study and tertiles of the protein at baseline (including a protein-by-treatment interaction term).

The geometric mean concentrations of BMP10 (in Cohort 1) and levels of BMP10, ALK1 and endoglin (in Cohort 2) were assessed at the 12- and 52-week visits using a mixed model for repeated measurements. For Cohort 1, BMP10 concentrations were log-transformed prior to fitting the model. The model was adjusted for age, estimated glomerular filtration rate, region, diabetes status, sex, study, left ventricular ejection fraction, and baseline protein values at each visit. Between-group differences were compared at each time point using the adjusted geometric mean ratio.

Results

Relation of BMP10/ALK1/endoglin levels and patient characteristics

Measurement of BMP10 by electrochemiluminescence immunoassay in Cohort 1

In Cohort 1, the median (Q1–Q3) concentrations of BMP10 at baseline in the pooled analysis was 2.71 ng/ml (2.20–3.37 ng/mL), and tertiles 1, 2, and 3 of BMP10 concentrations were <2.35 ng/ml, 2.35 to <3.11 ng/ml and ≥3.11 ng/ml, respectively.

As compared with patients who had lower levels, patients with higher concentrations of BMP10 were more likely to be female and older with a lower body mass index, and had more severe heart failure, as reflected by greater prevalence of New York Heart Association class III–IV symptoms, worse renal function and higher levels of natriuretic peptides and troponin T (p for trend <0.05 for all) (*Table 1*). However, there were only modest relationships between BMP10 concentrations and the concentrations of NT-proBNP and hs-cTnT at baseline (Spearman rho = 0.38 and 0.18, respectively). Interestingly, we observed a striking relationship between BMP10 concentrations and atrial fibrillation ($p < 0.0001$), with atrial fibrillation being present in ~30% of patients in the lowest

Table 1 Characteristics of participants by baseline bone morphogenetic protein 10 concentrations, as measured by electrochemiluminescence immunoassay (Cohort 1)

	Baseline BMP10 concentration (electrochemiluminescence immunoassay)			*p*-value for trend
	Tertile 1 (<2.35 ng/ml) (*n* = 371)	Tertile 2 (2.35–3.11 ng/ml) (*n* = 380)	Tertile 3 (≥3.11 ng/ml) (*n* = 376)	
Age, years	67.9 ± 10.3	70.7 ± 9.3	71.3 ± 10.2	<0.0001
Female sex, *n* (%)	99 (26.7)	123 (32.4)	139 (37.0)	0.003
Race/ethnicity, *n* (%)				<0.0001
Asian	17 (4.6)	29 (7.6)	49 (13.0)	
Black or African American	10 (2.7)	7 (1.8)	8 (2.1)	
White	318 (85.7)	324 (85.3)	309 (82.2)	
Other (including mixed race)	23 (6.2)	14 (3.7)	8 (2.1)	
Geographic region, *n* (%)				<0.0001
Asia-Pacific	13 (3.5)	26 (6.8)	46 (12.2)	
Europe	217 (58.5)	239 (62.9)	178 (47.3)	
North America	54 (14.6)	39 (10.3)	64 (17.0)	
Latin America	77 (20.8)	69 (18.2)	71 (18.9)	
Hospitalization for heart failure within 1 year, *n* (%)	94 (25.3)	105 (27.6)	111 (29.5)	0.20
Body mass index, kg/m^2	29.9 ± 5.2	29.5 ± 5.7	28.7 ± 6.0	0.003
Ejection fraction, %	39.2 ± 14.6	40.4 ± 14.7	40.6 ± 15.7	0.19
NYHA functional class[a], *n* (%)				0.004
II	316 (85.2)	301 (79.2)	286 (76.1)	
III–IV	55 (14.8)	79 (20.8)	90 (23.9)	
Systolic blood pressure, mmHg	128.6 ± 15.6	128.4 ± 15.9	126.7 ± 15.8	0.09
Heart rate, bpm	69.5 ± 11.3	69.3 ± 10.6	72.5 ± 13.1	0.0003
Cardiovascular comorbidities, *n* (%)				
Hypertension	302 (81.4)	315 (82.9)	317 (84.3)	0.29
Diabetes mellitus	173 (46.6)	187 (49.2)	184 (48.9)	0.53
Atrial fibrillation or atrial flutter	116 (31.3)	195 (51.3)	238 (63.3)	<0.001
Coronary artery disease	173 (46.6)	163 (42.9)	124 (33.0)	<0.001
Medications, *n* (%)				
ACEI, ARB or ARNI	339 (91.4)	337 (88.7)	304 (80.9)	<0.0001
Loop or thiazide diuretics	291 (78.4)	325 (85.5)	311 (82.7)	0.12
Beta-blockers	352 (94.9)	355 (93.4)	331 (88.0)	0.0004
Mineralocorticoid receptor antagonist	208 (56.1)	205 (53.9)	190 (50.5)	0.13
Estimated glomerular filtration rate, *n* (%)				<0.0001
≥60 ml/min/1.73 m^2	224 (60.4)	176 (46.3)	150 (39.9)	
<60 ml/min/1.73 m^2	147 (39.6)	204 (53.7)	226 (60.1)	
NT-proBNP[b] (pg/ml)	921 [533–1621]	1299 [730–2239]	2054 [1224–3559]	<0.0001
High-sensitivity troponin T[b] (ng/L)	17.5 [12.0–26.8]	18.5 [13.1–28.2]	23.7 [14.8–34.2]	<0.0001

Values are given as mean ± standard deviation, *n* (%), or median [interquartile range].
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor–neprilysin inhibitor; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.
[a]NYHA class II includes one patient with NYHA class I and NYHA class III–IV includes two patients with NYHA class IV.
[b]Analyses of NT-proBNP and troponin were based on log-transformed values.

BMP10 tertile and >60% of patients in the highest tertile. Left ventricular ejection fraction did not differ across BMP10 tertiles. These patterns were observed when EMPEROR-Reduced and EMPEROR-Preserved were analysed separately.

Measurements of BMP10/ALK1/endoglin by proximity extension assay in Cohort 2

The associations of BMP10 levels and baseline characteristics were confirmed in Cohort 2. Patients with higher BMP10 expression levels were more likely to be female and older with a lower body mass index, and had more severe heart failure, as reflected by greater prevalence of New York Heart Association class III–IV symptoms, worse renal function and higher levels of natriuretic peptides and troponin T (*p* for trend <0.05 for all) (*Table 2*). As in Cohort 1, we also observed a highly significant relationship between BMP10 expression levels and atrial fibrillation ($p < 0.0001$). Additionally, patients with higher expression levels of ALK1 and of endoglin were older and were more likely to have more severe heart failure, atrial fibrillation, and higher levels of natriuretic peptides and troponin T (*p* for trend <0.05 for all) (*Tables 3* and *4*).

Table 2 Characteristics of participants by baseline bone morphogenetic protein 10 levels, as measured by proximity extension assay (Cohort 2)

Characteristics	Baseline BMP10 level (proximity extension assay)			*p*-value for trend
	Tertile 1 (*n* = 369)	Tertile 2 (n = 369)	Tertile 3 (*n* = 370)	
Age, years	68.7 ± 9.8	70.8 ± 9.1	71.1 ± 10.0	0.0008
Female sex, *n* (%)	86 (23.3)	126 (34.1)	135 (36.5)	0.0001
Race/ethnicity, *n* (%)				0.78
Asian	25 (6.8)	31 (8.4)	32 (8.6)	
Black or African American	7 (1.9)	9 (2.4)	9 (2.4)	
White	320 (86.7)	312 (84.6)	314 (84.9)	
Other (including mixed race)	12 (3.3)	15 (4.1)	12 (3.2)	
Geographic region, *n* (%) < 0.001				0.68
Asia-Pacific	22 (6.0)	28 (7.6)	29 (7.8)	
Europe	226 (61.2)	229 (62.1)	224 (60.5)	
North America	44 (11.1)	37 (10.0)	40 (10.8)	
Latin America	73 (19.8)	68 (18.4)	66 (17.8)	
Hospitalization for heart failure within 1 year, *n* (%)	92 (24.9)	106 (28.7)	103 (27.8)	0.38
Body mass index, kg/m^2	30.1 ± 5.3	29.7 ± 5.5	28.9 ± 5.9	0.002
Ejection fraction, %	40.0 ± 15.0	39.8 ± 14.6	40.2 ± 15.3	0.85
NYHA functional class[a], *n* (%)				0.001
II	310 (84.0)	294 (79.7)	272 (73.5)	
III–IV	59 (16.0)	75 (20.3)	98 (26.5)	
Systolic blood pressure, mmHg	127.9 ± 15.8	129.2 ± 16.5	128.0 ± 16.4	0.94
Heart rate, bpm	69.2 ± 11.0	70.8 ± 11.4	72.8 ± 13.0	< 0.0001
Cardiovascular comorbidities, *n* (%)				
Hypertension	315 (85.4)	306 (82.9)	317 (85.7)	0.91
Diabetes mellitus	181 (49.1)	196 (53.1)	184 (49.7)	0.85
Atrial fibrillation or atrial flutter	145 (39.3)	179 (48.5)	220 (59.5)	<0.0001
Coronary artery disease	168 (45.5)	152 (41.2)	145 (39.2)	0.08
Medications, *n* (%)				
ACEI, ARB or ARNI	334 (90.5)	313 (84.8)	300 (81.1)	0.0003
Loop or thiazide diuretics	308 (83.5)	325 (85.5)	324 (87.6)	0.12
Beta-blockers	350 (94.9)	340 (92.1)	333 (90.0)	0.014
Mineralocorticoid receptor antagonist	207 (56.1)	201 (54.5)	212 (57.3)	0.74
Estimated glomerular filtration rate, *n* (%)				<0.0001
≥60 ml/min/1.73 m^2	220 (59.6)	190 (51.5)	161 (43.5)	
<60 ml/min/1.73 m^2	148 (40.1)	179 (48.5)	209 (56.5)	
NT-proBNP[b] (pg/ml)	1048 [564–1791]	1170 [690–2265]	1967 [1001–3538]	<0.0001
High-sensitivity troponin T[b] (ng/L)	17.4 [12.1–24.7]	19.7 [13.6–32.0]	23.2 [14.9–34.3]	<0.0001

Values are given as mean ± standard deviation, *n* (%), or median [interquartile range].
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.
[a]NYHA class II includes one patient with NYHA class I, and NYHA class III–IV includes two patients with NYHA class IV.
[b]Analyses of NT-proBNP and troponin were based on log-transformed values.

Considered individually, in Cohort 2, as BMP10 expression levels increased, expression levels of ALK1 and endoglin increased. There were meaningful linear relationships (1) between BMP10 and ALK1 (Spearman rho = 0.48) and with endoglin (Spearman rho = 0.61) at baseline; (2) between changes in BMP10 and changes in ALK1 and changes in endoglin at 12 weeks (Spearman rho in empagliflozin group = 0.62 and 0.73, respectively; $p < 0.0001$ for both); and (3) between changes in BMP10 and changes in ALK1 and changes in endoglin at 52 weeks (Spearman rho in empagliflozin group = 0.67 and 0.77, respectively; $p < 0.0001$ for both), (*Figures 1* and *2*).

Relation of BMP10, ALK1 and endoglin levels and clinical course

In Cohort 1, when measured by electrochemiluminescence immunoassay, higher baseline concentrations of BMP10 were asso-

Table 3 Characteristics of participants by baseline activin receptor-like kinase 1 levels, as measured by proximity extension assay (Cohort 2)

Characteristics	Baseline ALK1 level (proximity extension assay)			*p*-value for trend
	Tertile 1 (*n* = 354)	Tertile 2 (*n* = 354)	Tertile 3 (*n* = 355)	
Age, years	68.3 ± 9.9	70.3 ± 9.7	71.8 ± 9.3	<0.0001
Female sex, *n* (%)	107 (30.2)	115 (32.5)	111 (31.3)	0.77
Race/ethnicity, *n* (%)				0.02
Asian	37 (10.5)	29 (8.2)	19 (5.4)	
Black or African American	3 (0.8)	10 (2.8)	11 (3.1)	
White	299 (84.5)	297 (83.9)	307 (86.5)	
Other (including mixed race)	11 (3.1)	17 (4.8)	13 (3.7)	
Geographic region, *n* (%)				0.02
Asia Pacific	36 (10.2)	25 (7.1)	15 (4.2)	
Europe	213 (60.2)	208 (58.8)	227 (63.9)	
North America	32 (9.0)	38 (10.7)	46 (13.0)	
Latin America	65 (18.4)	74 (20.9)	60 (16.9)	
Other	8 (2.3)	9 (2.5)	7 (2.0)	
Hospitalization for heart failure within 1 year, *n* (%)	87 (24.6)	101 (28.5)	100 (28.2)	0.28
Body mass index, kg/m^2	29.5 ± 5.4	29.5 ± 5.2	29.5 ± 5.9	0.88
Ejection fraction, %	41.0 ± 14.9	40.4 ± 14.8	39.2 ± 15.0	0.11
NYHA functional class[a], *n* (%)				
II	304 (85.9)	284 (80.2)	258 (72.4)	0.004
III–IV	50 (14.1)	70 (19.8)	96 (27.3)	
Systolic blood pressure, mmHg	129.0 ± 15.6	128.6 ± 15.9	128.1 ± 17.2	0.46
Heart rate, bpm	69.4 ± 10.7	70.7 ± 11.7	72.5 ± 13.4	< 0.0001
Cardiovascular comorbidities, *n* (%)				
Hypertension	290 (81.9)	303 (85.6)	308 (86.8)	0.07
Diabetes mellitus	160 (45.2)	171 (48.3)	206 (58.0)	0.0006
Atrial fibrillation or atrial flutter	139 (39.3)	182 (51.4)	199 (56.1)	<0.0001
Coronary artery disease	132 (37.3)	147 (41.5)	166 (46.8)	0.01
Medications for heart failure, *n* (%)				
ACEI, ARB or ARNI	321 (90.7)	297 (83.9)	292 (82.3)	0.002
Loop or thiazide diuretics	279 (78.8)	303 (85.6)	318 (89.6)	<0.0001
Beta-blockers	330 (93.2)	326 (92.1)	334 (94.1)	0.48
Mineralocorticoid receptor antagonist	184 (52.0)	201 (56.8)	200 (56.3)	0.24
Estimated glomerular filtration rate, *n* (%)				<0.0001
≥60 ml/min/1.73 m^2	272 (76.8)	193 (54.5)	86 (24.2)	
<60 ml/min/1.73 m^2	82 (23.2)	160 (45.2)	269 (75.8)	
NT-proBNP[b] (pg/mL), median (IQR)	952 [534–1684]	1253 [691–2316]	1947 [994–3649]	<0.0001
High-sensitivity troponin T[b] (ng/L), median (IQR)	15.2 [10.5–22.2]	19.9 [13.8–28.6]	25.4 [17.6–39.3]	<0.0001

Values are given as mean ± standard deviation, *n* (%), or median [interquartile range].
ACEI, angiotensin-converting enzyme inhibitor; ALK1, activin receptor-like kinase 1; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.
[a]NYHA class II includes one patient with NYHA class I and NYHA class III–IV includes one patient with NYHA class IV.
[b]Analyses of NT-proBNP and troponin were based on log-transformed values.

ciated with a higher risk of cardiovascular death or hospitalization for heart failure, particularly in patients in the highest BMP10 tertile (7.0, 6.8, and 16.3 events per 100 patient-years at risk for <2.35 ng/ml, 2.35–<3.11 ng/ml and ≥3.11 ng/ml, respectively; *p* for trend <0.0001) (*Table 5* and *Figure 3*). A similar pattern of risk was seen for time-to-first hospitalization, total heart failure hospitalizations, cardiovascular death, and all-cause mortality (*Table 5*).

In Cohort 2, when measured by proximity extension assay, we observed a significant linear relationship between baseline expression levels of BMP10 and the occurrence of cardiovascular death or hospitalization for heart failure, with hazard ratios and *p* for trend tests that were remarkably similar to those seen when BMP10 was measured by electrochemiluminescence immunoassay in Cohort 1 (*Table 5* and *Figure 3*). The pattern of stepwise associations of BMP10 expression levels and cardiovascular death or hospitalizations for heart failure (and other major heart failure outcomes) seen in Cohort 1 was confirmed in Cohort 2.

Additionally, we observed stepwise associations of baseline ALK1 and endoglin expression levels and the primary endpoint and with hospitalizations for heart failure (time-to-event or first and recurrent events) in Cohort 2 (*Table 6* and *Figure 3*). The patterns

Table 4 Characteristics of participants by baseline endoglin levels, as measured by proximity extension assay (Cohort 2)

Characteristics	Baseline endoglin level (proximity extension assay)			*p*-value for trend
	Tertile 1 (*n* = 369)	Tertile 2 (*n* = 370)	Tertile 3 (*n* = 370)	
Age, years	71.3 ± 8.9	69.9 ± 9.9	69.5 ± 10.1	0.01
Female sex, *n* (%)	115 (31.2%)	116 (31.4%)	116 (31.4%)	0.96
Race/ethnicity, *n* (%)				0.28
Asian	34 (9.2)	33 (8.9)	20 (5.4)	
Black or African American	7 (1.9)	10 (2.7)	7 (1.9)	
White	311 (84.3)	307 (83.0)	329 (88.9)	
Other (including mixed race)	11 (3.0)	20 (5.4)	10 (2.7)	
Geographic region, *n* (%)				0.07
Asia Pacific	32 (8.7)	29 (7.8)	17 (4.6)	
Europe	238 (64.5)	209 (56.5)	234 (63.2)	
North America	39 (10.6)	43 (11.6)	34 (9.2)	
Latin America	54 (14.6)	79 (21.4)	75 (20.3)	
Other	7 (1.6)	10 (2.7)	10 (2.7)	
Hospitalization for heart failure within 1 year, *n* (%)	97 (26.3)	100 (27.0)	102 (27.6)	0.70
Body mass index, kg/m^2	29.5 ± 5.1	29.8 ± 5.6	29.5 ± 6.0	0.92
Ejection fraction, %	39.3 ± 14.8	41.5 ± 14.9	40.0 ± 15.0	0.49
NYHA functional class[a], *n* (%)				<0.0001
II	309 (83.7)	299 (80.8)	266 (71.9)	
III–IV	60 (16.3)	71 (19.2)	104 (28.1)	
Systolic blood pressure, mmHg	128.2 ± 16.1	129.0 ± 16.4	128.1 ± 16.2	0.89
Heart rate, bpm	69.0 ± 10.8	70.5 ± 10.9	72.9 ± 13.4	< 0.0001
Cardiovascular comorbidities, *n* (%)				
Hypertension	317 (85.9)	315 (85.1)	308 (83.2)	0.31
Diabetes mellitus	191 (51.8)	181 (48.9)	189 (51.1)	0.85
Atrial fibrillation or atrial flutter	159 (43.1)	167 (45.1)	218 (58.9)	0.0001
Coronary artery disease	173 (46.9)	140 (37.8)	151 (40.8)	0.09
Medications for heart failure, *n* (%)				
ACEI, ARB or ARNI, *n* (%)	334 (90.5)	312 (84.3)	303 (81.9)	0.0009
Loop or thiazide diuretics	308 (83.5)	315 (85.1)	318 (85.9)	0.35
Beta-blockers	342 (92.7)	348 (94.1)	334 (90.3)	0.21
Mineralocorticoid receptor antagonist	200 (54.2)	201 (54.3)	215 (58.1)	0.29
Estimated glomerular filtration rate, *n* (%)				0.58
≥60 ml/min/1.73 m^2	181 (49.1)	203 (54.9)	189 (51.1)	
<60 ml/min/1.73 m^2	188 (50.9)	166 (44.9)	181 (48.9)	
NT-proBNP[b] (pg/ml)	1167 [670–2062]	1115 [609–2092]	1853 [947–3232]	<0.0001
High-sensitivity troponin T[b] (ng/L)	20.0 [13.2–28.3]	19.0 [13.4–28.3]	21.8 [14.0–33.7]	0.02

Values are given as mean ± standard deviation, *n* (%), or median [interquartile range].
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.
[a]NYHA class II includes one patient with NYHA class I and NYHA class III–IV includes one patient with NYHA class IV.
[b]Analyses of NT-proBNP and troponin were based on log-transformed values.

of the cumulative incidence curves for the influence of baseline BMP10, ALK1 and endoglin levels were strikingly similar to each other (*Figure 3*). Specifically, for each of the three proteins, the risks in the bottom two tertiles were similar to each other, but in contrast, the risk of an event in the patients in the highest tertile was two- to three-fold greater than in the patients in the lowest two tertiles. However, in contrast with the findings with BMP10 in Cohort 1, the associations between BMP10, ALK1 and endoglin expression levels and cardiovascular death and all-cause mortality were of borderline statistical significance in Cohort 2.

Interactions of empagliflozin and BMP10, ALK1 and endoglin levels

In Cohort 1, treatment with empagliflozin treatment was accompanied with a significant reduction in BMP10 versus placebo at 12 weeks (adjusted geometric mean ratio 0.94 [0.91–0.97], $p < 0.001$) (based on 1116 patients), but no effect was observed at week 52 (1.00 [0.96–1.04], $p = 0.86$) (based on 710 patients) (*Figure 4*). Similar results were seen when EMPEROR-Reduced and EMPEROR-Preserved cohorts were analysed separately.

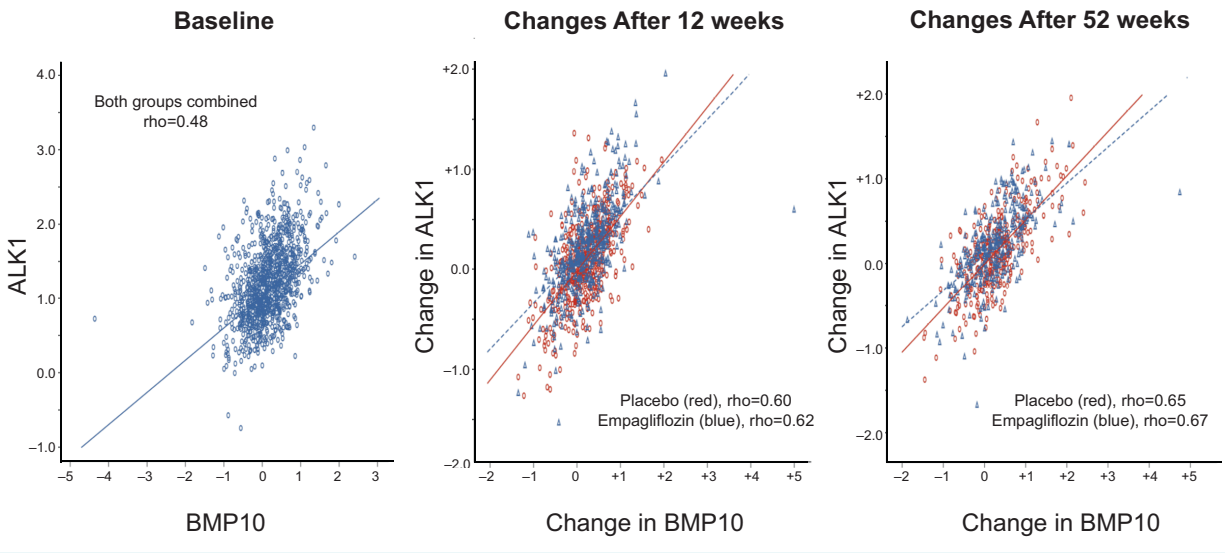

Figure 1 Correlations between bone morphogenetic protein 10 (BMP10) and activin receptor-like kinase 1 (ALK1) expression levels at baseline, and between changes in BMP10 and changes in ALK1 expression levels after 12 and 52 weeks in Cohort 2. There were meaningful linear relationships (1) between BMP10 and ALK1 at baseline (Spearman rho = 0.48) (*A*); (2) between changes in BMP10 and changes in ALK1 at 12 weeks (Spearman rho = 0.60 for placebo group and 0.62 for empagliflozin group) (*B*); and (3) between changes in BMP10 and changes in ALK1 at 52 weeks (Spearman rho = 0.65 for placebo group and 0.67 for empagliflozin group) (*C*) ($p < 0.0001$ for all).

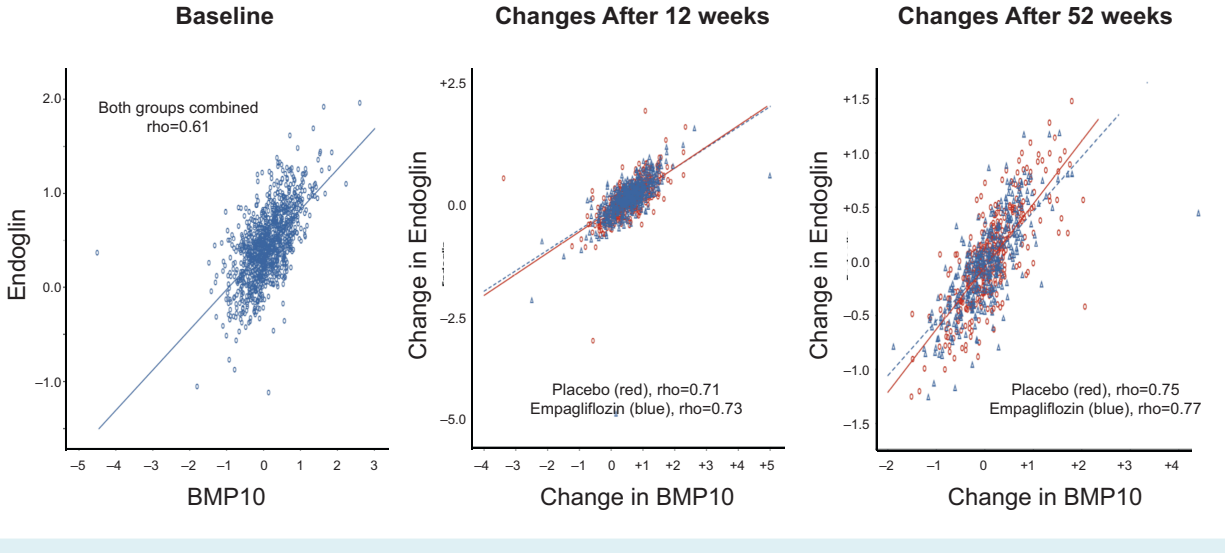

Figure 2 Correlations between bone morphogenetic protein 10 (BMP10) and endoglin expression levels at baseline, and between changes in BMP10 and changes in endoglin expression levels after 12 and 52 weeks in Cohort 2. There were meaningful linear relationships (1) between BMP10 and endoglin at baseline (Spearman rho = 0.61) (*A*); (2) between changes in BMP10 and changes in endoglin at 12 weeks (Spearman rho = 0.71 for placebo group and 0.73 for empagliflozin group) (*B*); and (3) between changes in BMP10 and changes in endoglin at 52 weeks (Spearman rho = 0.75 for placebo group and 0.77 for empagliflozin group) (*C*) ($p < 0.0001$ for all).

Table 5 Major heart failure outcome according to baseline bone morphogenetic protein 10 concentration, as measured by electrochemiluminescence immunoassay (Cohort 1) and by proximity extension assay (Cohort 2)

Cohort 1 (discovery cohort)	Baseline BMP10 concentration (electrochemiluminescence immunoassay)			*p*-value for trend
	Tertile 1 (<2.35 ng/ml) (*n* = 371)	Tertile 2 (2.35–3.11 ng/ml) (*n* = 380)	Tertile 3 (≥3.11 ng/ml) (*n* = 376)	
Cardiovascular death or hospitalization for heart failure				
Number with event (rate per 100 patient-years)	43 (7.0)	43 (6.8)	91 (16.3)	<0.0001
Hazard ratio (95% CI), compared to Tertile 1		0.92 (0.60–1.41)	2.16 (1.48–3.15)	
Time to first hospitalization for heart failure				
Number with event (rate per 100 patient-years)	27 (4.4)	32 (5.1)	69 (12.3)	<0.0001
Hazard ratio (95% CI), compared to Tertile 1		1.09 (0.65–1.83)	2.47 (1.55–3.92)	
Total hospitalizations for heart failure				
Number of events (rate per 100 patient-years)	40 (6.3)	49 (7.5)	127 (20.4)	<0.0001
Hazard ratio (95% CI), compared to Tertile 1		1.01 (0.59–1.77)	2.83 (1.68–4.76)	
Cardiovascular death				
Number with event (rate per 100 patient-years)	22 (3.5)	19 (2.9)	42 (6.7)	0.009
Hazard ratio (95% CI), compared with Tertile 1		0.79 (0.43–1.47)	1.96 (1.14–3.36)	
All-cause mortality				
Number with event (rate per 100 patient-years)	29 (4.6)	32 (4.9)	60 (9.6)	0.0003
Hazard ratio (95% CI), compared with Tertile 1		1.02 (0.61–1.69)	2.23 (1.40–3.54)	
Cohort 2 (validation cohort)	**Baseline BMP10 level (proximity extension assay)**			***p*-value for trend**
	Tertile 1 (*n* = 369)	**Tertile 2 (*n* = 369)**	**Tertile 3 (*n* = 370)**	
Cardiovascular death or hospitalization for heart failure16.3)				
Number with event (rate per 100 patient-years)	42 (7.1)	53 (8.5)	82 (14.2)	0.0001
Hazard ratio (95% CI), compared to Tertile 1		1.20 (0.79–1.81)	2.03 (1.39–2.97)	
Time to first hospitalization for heart failure				
Number with event (rate per 100 patient-years)	30 (5.1)	34 (5.6)	59 (10.2)	0.0007
Hazard ratio (95% CI), compared to Tertile 1		1.11 (0.67–1.82)	2.08 (1.33–3.26)	
Total hospitalizations for heart failure				
Number of events (rate per 100 patient-years)	38 (6.2)	55 (8.5)	100 (15.9)	<0.0001
Hazard ratio (95% CI), compared to Tertile 1		1.41 (0.83–2.40)	3.22 (1.93–5.39)	
Cardiovascular death				
Number with event (rate per 100 patient-years)	20 (3.2)	25 (3.8)	36 (5.7)	0.06
Hazard ratio (95% CI), compared with Tertile 1		1.13 (0.63–2.06)	1.67 (0.95–2.96)	
All-cause mortality				
Number with event (rate per 100 patient-years)	30 (4.9)	43 (6.6)	44 (6.9)	0.27
Hazard ratio (95% CI), compared with Tertile 1		1.28 (0.79–2.05)	1.32 (0.82–2.13)	

BMP10, bone morphogenetic protein 10; CI, confidence interval.

Conversely, when measured by proximity extension assay in Cohort 2, empagliflozin had no significant effect on BMP10 levels at 12 weeks (ratio of treatment arms 0.99 [0.96–1.03], $p = 0.67$), but empagliflozin had a significant (but modest) effect to reduce BMP10 levels at 52 weeks (ratio of treatment arms 0.95 [0.91–0.99], $p = 0.01$) (*Figure 4*). Empagliflozin had a modest effect on ALK1 expression levels at 12 weeks (1.070 [1.04–1.10]) but not at 52 weeks, and the drug had no significant effect on endoglin expression levels either at 12 or 52 weeks.

When measured in Cohort 1 or Cohort 2, the effect of empagliflozin to reduce major heart failure events or cardiovascular death did not differ across the tertiles of baseline BMP10 concentrations or expression levels (online supplementary *Tables S1* and *S2*). When measured in Cohort 2, the effect of empagliflozin to reduce major heart failure events or cardiovascular death did not differ across the tertiles of baseline BMP10 or ALK1; however, the effect of empagliflozin to reduce first and total hospitalizations for heart failure appeared to be most apparent in patients with the lowest endoglin expression levels (p for trend = 0.08).

Discussion

BMP10, ALK1 and endoglin form a TGF-β family signalling complex that plays a critical role in cardiac and vascular morphogenesis and maturation during hypoxic embryonic development, but these proteins are typically down-regulated with the onset of normoxia at the time of birth.[18,19,26,27,47,48] Following cardiac injury in the adult heart, the up-regulation of BMP10/ALK1/endoglin triggers a molecular switch that may allow TGF-β family members to activate pro-hypertrophic and pro-fibrotic pathways.[29,30,56,57]

The current study is the first to simultaneously measure circulating levels of BMP10, ALK1 and endoglin in patients with heart

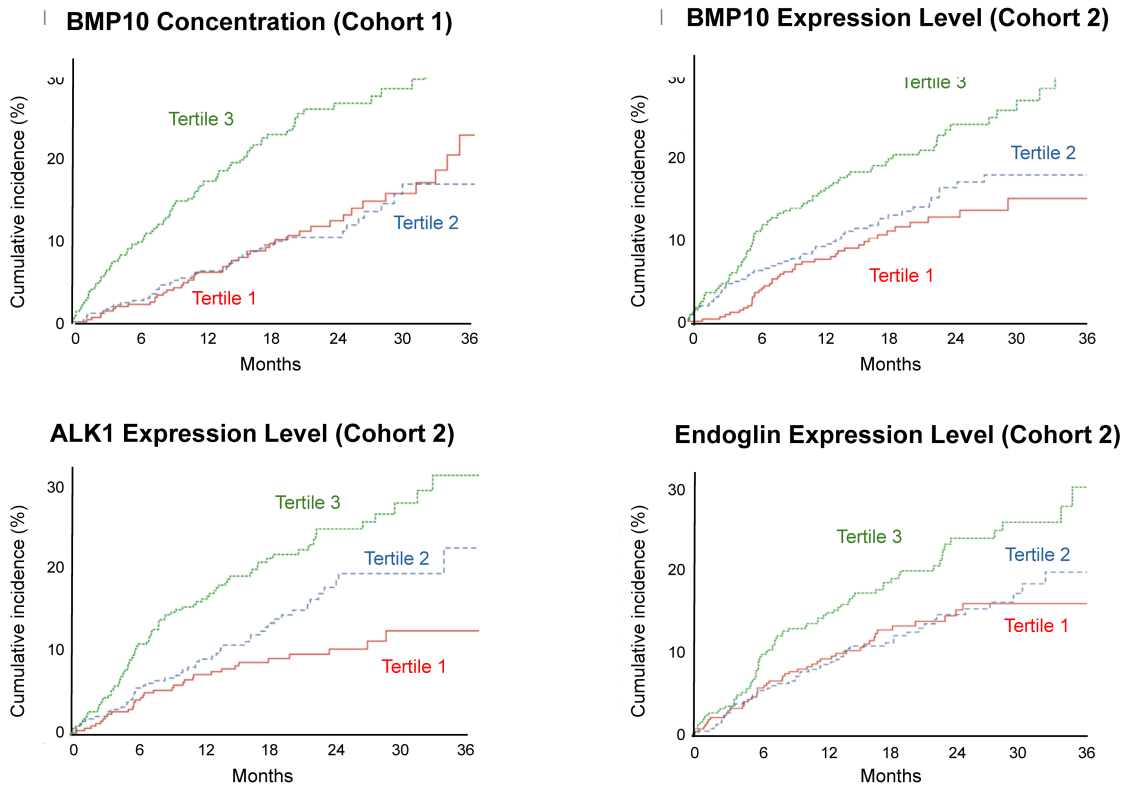

Figure 3 Relationship between baseline levels of bone morphogenetic protein 10 (BMP10), activin receptor-like kinase 1 (ALK1) and endoglin and the subsequent risk of cardiovascular death or hospitalization for heart failure (primary endpoint). Shown are the cumulative incidence plots for the risk of cardiovascular death and hospitalization for heart failure for tertiles 1, 2 and 3 for baseline BMP10 concentrations in Cohort 1 (*A*) and baseline BMP10 expression levels in Cohort 2 (*B*), baseline ALK1 expression levels in Cohort 2 (*C*) and baseline endoglin expression levels in Cohort 2 (*D*).

failure, and we found that levels of BMP10, ALK1 and endoglin are all increased in proportion to the severity of heart failure. Higher levels of BMP10, ALK1 and endoglin identified patients with greater severity of symptoms, worse renal function and higher levels of NT-proBNP and troponin, the primary biomarkers of ventricular stretch and injury in patients with heart failure. Our findings with BMP10 measured by electrochemiluminescence immunoassay in Cohort 1 were confirmed with BMP10 levels measured by proximity extension assay in our Cohort 2. Furthermore, circulating levels of BMP10, ALK1 and endoglin were closely correlated with each other at baseline, and importantly, changes in the levels of these three proteins were closely correlated with each other after 12 and 52 weeks. In contrast, BMP10 levels were not closely related to NT-proBNP or troponin at baseline or during the course of follow-up. These observations are consistent with an underlying mechanism that drives highly coordinated BMP10, ALK1 and endoglin signalling, which becomes progressively more intense with the progression of heart failure.

Patients with levels in the highest tertiles of BMP10, ALK1 and endoglin had the most unfavourable prognosis, as reflected by markedly increased risk of cardiovascular death or hospitalization for heart failure and other major heart failure outcomes. The patterns of the cumulative incidence curves for the influence of BMP10, ALK1 and endoglin on the primary endpoint were strikingly similar to each other; specifically, the bottom two tertiles showed a similar risk, whereas the risks in patients in the highest tertiles were two–three-fold greater than in patients in the two lowest tertiles. Our findings with BMP10 measured by electrochemiluminescence immunoassay in Cohort 1 were confirmed with BMP10 levels measured by proximity extension assay in Cohort 2, with similar clinical features and risk estimates for increasing levels of BMP10 in the two cohorts. One previous study reported prognostic significance for BMP10 measurements in patients with heart failure, but it did not evaluate the two other components of the signalling complex.[44] Taken collectively, these clinical findings are consistent with experimental studies that support progressive intensity of BMP10, ALK1 and endoglin signalling as heart failure progresses.

In addition, our results confirm prior work showing a strong association between BMP10 and atrial fibrillation. In the healthy heart, BMP10 expression is limited to the right atrium, with selective down-regulation of BMP10 in the left atrium.[61,62] Failure of left atrial BMP10 down-regulation results in atrial structural abnormalities,[63] and thus, it is not surprising that any expansion of

Table 6 Major heart failure outcome according to baseline activin receptor-like kinase 1 and endoglin levels, as measured by proximity extension assay (Cohort 2)

	Baseline ALK1 levels (proximity extension assay)			*p*-value for trend
	Tertile 1 (*n* = 354)	Tertile 2 (*n* = 354)	Tertile 3 (*n* = 355)	
Cardiovascular death or hospitalization for heart failure				
Number with event (rate per 100 patient-years)	34 (5.8)	52 (9.2)	81 (14.7)	0.0004
Hazard ratio (95% CI), compared to Tertile 1		1.51 (0.97–2.35)	2.27 (1.43–3.59)	
Time to first hospitalization for heart failure				
Number with event (rate per 100 patient-years)	24 (4.0)	33 (5.9)	60 (10.9)	0.0004
Hazard ratio (95% CI), compared to Tertile 1		1.42 (0.82–2.44)	2.61 (1.50–4.53)	
Total hospitalizations for heart failure				
Number of events (rate per 100 patient-years)	38 (6.0)	53 (8.9)	94 (15.6)	<0.0001
Hazard ratio (95% CI), compared to Tertile 1		1.64 (0.93–2.88)	4.14 (2.23–7.69)	
Cardiovascular death				
Number with event (rate per 100 patient-years)	16 (2.5)	28 (4.6)	33 (5.5)	0.17
Hazard ratio (95% CI), compared with Tertile 1		1.54 (0.83–2.95)	1.65 (0.83–3.28)	
All-cause mortality				
Number with event (rate per 100 patient-years)	23 (3.6)	39 (6.5)	50 (8.3)	0.08
Hazard ratio (95% CI), compared with Tertile 1		1.54 (0.91–2.63)	1.71 (0.96–3.03)	
	Baseline endoglin levels (proximity extension assay)			***p*-value for trend**
	Tertile 1 (*n* = 369)	**Tertile 2 (*n* = 370)**	**Tertile 3 (*n* = 370)**	
Cardiovascular death or hospitalization for heart failure				
Number with event (rate per 100 patient-years)	48 (8.2)	51 (8.1)	76 (13.4)	0.003
Hazard ratio (95% CI), compared to Tertile 1		1.10 (0.74–1.64)	1.71 (1.19–2.47)	
Time to first hospitalization for heart failure				
Number with event (rate per 100 patient-years)	38 (6.5)	32 (5.0)	53 (9.3)	0.04
Hazard ratio (95% CI), compared to Tertile 1		0.87 (0.54–1.40)	1.52 (1.00–2.32)	
Total hospitalizations for heart failure				
Number of events (rate per 100 patient-years)	56 (9.1)	49 (7.4)	88 (14.2)	0.001
Hazard ratio (95% CI), compared to Tertile 1		0.93 (0.55–1.54)	2.22 (1.35–3.65)	
Cardiovascular death				
Number with event (rate per 100 patient-years)	21 (3.4)	25 (3.7)	34 (5.5)	0.08
Hazard ratio (95% CI), compared with Tertile 1		1.18 (0.65–2.12)	1.63 (0.94–2.82)	
All-cause mortality				
Number with event (rate per 100 patient-years)	34 (5.5)	35 (5.2)	47 (7.6)	0.09
Hazard ratio (95% CI), compared with Tertile 1		1.00 (0.62–1.62)	1.46 (0.93–2.28)	

ALK1, activin receptor-like kinase 1; CI, confidence interval.

BMP10 signalling in the adult heart exerts a pro-fibrotic action that preferentially affects left atrial tissue.[41] There exists a strong association of BMP10 and atrial fibrillation in patients with and without heart failure,[44,64,65] and BMP10 levels predict the occurrence of ischaemic stroke in patients with atrial fibrillation[43,44] and the recurrence of atrial fibrillation after catheter ablation.[66–70] In the present analysis, patients with heart failure who had BMP10 levels in the highest tertile were twice as likely to have atrial fibrillation than patients with BMP10 levels in the lowest tertile. We noted a similar association between levels of ALK1 and of endoglin with atrial fibrillation; these associations have not been previously reported either experimentally or clinically. Taken collectively, these findings support the hypothesis that BMP10/ALK1/endoglin signalling in the adult heart may promote both atrial and ventricular fibrosis, leading to atrial fibrillation and heart failure, respectively.

Experimental studies have demonstrated that SGLT2 inhibitors can exert anti-fibrotic effects,[71] but the mechanism of this action is likely to be multifactorial. SGLT2 inhibitors act to down-regulate HIF-1α,[72,73] the upstream effector for BMP10, and our observation that empagliflozin can modestly reduce levels of BMP10 at 12 weeks in Cohort 1 and at 52 weeks in Cohort 2 is consistent with an action of SGLT2 inhibitors to suppress signalling through HIF-1α. A reduction in the BMP10 ligand may possibly explain why we noted a small increase in circulating expression levels of ALK1 with empagliflozin, but SGLT2 inhibition was not associated with meaningful changes in endoglin, although the effect of empagliflozin

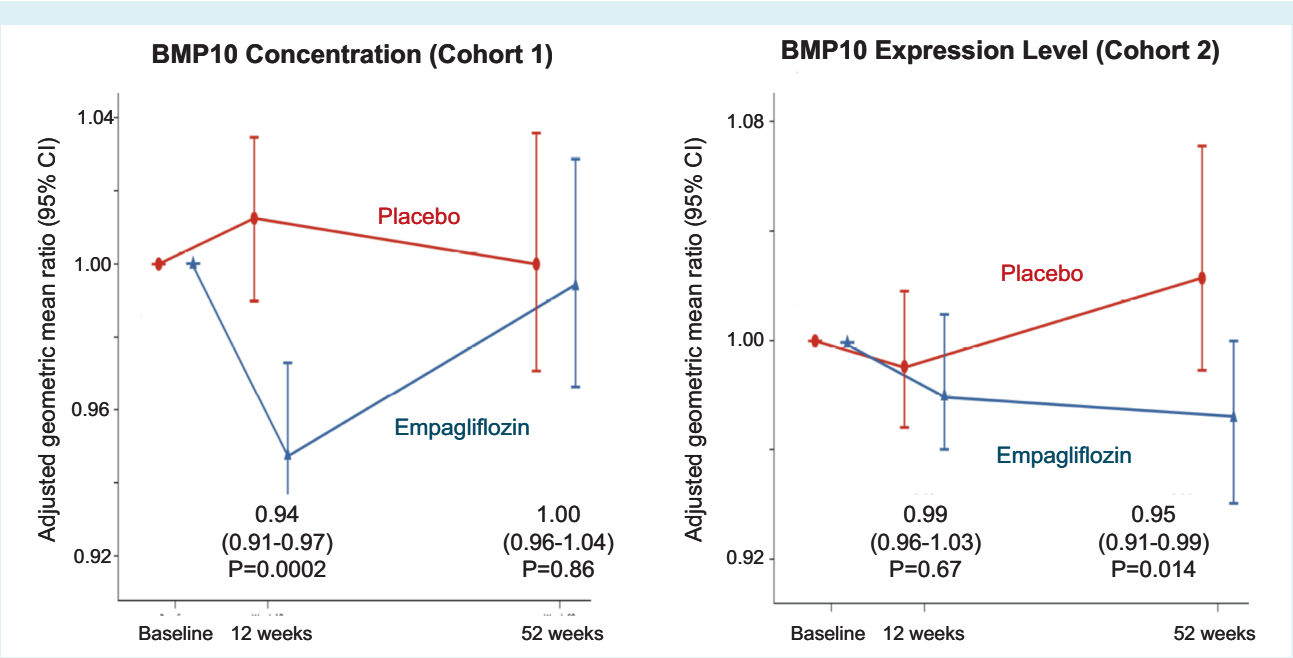

Figure 4 Effects of emplagliflozin and placebo on bone morphogenetic protein 10 (BMP10), assessed by electrochemiluminescence immunoassay after 12 and 52 weeks (*left panel*) and by proximity extension assay after 12 and 52 weeks (*right panel*). Shown are adjusted geometric ratio ratios with 95% confidence intervals. *P*-values indicate the level of statistical significance of the change from baseline.

to reduce hospitalizations for heart failure may have been most apparent in patients with the lowest endoglin expression levels. Given the multiplicity of comparisons, the significance of these changes is uncertain; however, the up-regulation of endoglin during experimental mechanical stress may be SGLT2-dependent,[55] and it is noteworthy that the pro-fibrotic effects of enhanced endoglin signalling may be mediated through Smad2/3,[74,75] and SGLT2 inhibitors can mute up-regulation Smad2/3 signalling, in parallel with a demonstrable antifibrotic effect.[76–80]

The results of our study should be interpreted in light of its strengths and limitations. Our findings regarding the clinical correlates and prognostic significance of circulating levels of BMP10 in heart failure are similar to those reported by Ceelen *et al.*[44] using electrochemiluminescence immunoassay in a single cohort of patients, but we performed our measurements using two different analytical methodologies in two distinct cohorts. Furthermore, our study is the first to characterize the clinical correlates and prognostic significance of ALK1 and endoglin in heart failure; prior studies of endoglin did not evaluate patients with established heart failure, were small and did not measure outcomes.[50,58] However, it should be emphasized that circulating blood-borne biomarkers may be poorly positioned to assess the biological meaningfulness of intracellular pathways, although it should be noted that BMP10, ALK1 and endoglin act primarily on the surfaces of cells. Nevertheless, our measurements assume that the shedding of these proteins from cell surfaces into the circulation is proportional to their biological effects, but the validity of this assumption is not known, and even if true, it is not clear that the heart is the major source of the proteins that we measured.

In conclusion, in this first large-scale characterization of the BMP10, ALK1 and endoglin complex in patients with heart failure, we found that patients who showed the most marked coordinated expression of these foetal cardiac morphogenetic proteins had more severe symptoms, greater evidence of cardiac stretch and injury, and the highest risk of the composite of cardiovascular death and hospitalization for heart failure. Up-regulation of BMP10, ALK1 and endoglin was closely aligned with each other at baseline and throughout follow-up, but not closely related to NT-proBNP or troponin, and the clinical and prognostic findings with BMP10 were demonstrated in two distinct cohorts using two different assays. Taken together, our observations are consistent with experimental data that cardiac injury and stress trigger the coordinated re-expression of key foetal proteins involved in cardiac and vascular morphogenesis, which are normally suppressed in the healthy adult heart. Further work is needed to determine if this pattern of foetal protein expression represents an adaptive or maladaptive response in patients with heart failure.

Supplementary Information

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

Funding

The study was supported and funded by Boehringer Ingelheim & Eli Lilly and Company Alliance. The assessment of BMP10 by an electrochemiluminescence immunoassay was performed within Biomarker Research Agreement of Boehringer Ingelheim and Roche Diagnostics International Ltd.

Conflict of interest: M.P. reports consulting fees from Abbvie, Actavis, Alnylam, Amgen, Amarin, ARMGO, AstraZeneca, Attralus, Biopeutics, Boehringer Ingelheim, Casana, CSL Behring, Cytokinetics Eli Lilly and Company, Moderna, Novartis, Pharmacosmos, Regeneron, and Salamandra. J.B. reports payment or honoraria for lectures, presentations, speaker bureaus, or educational events from Abbott, Adrenomed, Amgen, Applied Therapeutics, Array, AstraZeneca, Bayer, BerlinCures, Cardior, CVRx, Foundry, G3 Pharma, Imbria, Impulse Dynamics, Innolife, Janssen, LivaNova, Luitpold, Medtronic, Merck, Novartis, Novo Nordisk, Relypsa, Roche, Sanofi, Sequana Medical, Occlutech, and Vifor. J.P.F. reports consulting fees from Boehringer Ingelheim. J.L.J. is a trustee of the American College of Cardiology, a board member of Imbria Pharmaceuticals, has received grant support from Applied Therapeutics, Innolife, Novartis Pharmaceuticals, and Abbott Diagnostics, consulting income from Abbott, Janssen, Novartis, and Roche Diagnostics, and participates in clinical endpoint committees/data safety monitoring boards for Abbott, AbbVie, Amgen, Bayer, CVRx, Janssen, MyoKardia, and Takeda. N.S. reports personal fees from Amgen, AstraZeneca, Boehringer Ingelheim, Eli-Lilly, MSD, Novo Nordisk, Pfizer, and Sanofi, and grant income from Boehringer Ingelheim. S.G.M. is an employee of Roche Diagnostics. M.P.N. and M.S. are employees of Boehringer Ingelheim, the manufacturer of empagliflozin. S.J.P. reports consulting fees and payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Boehringer Ingelheim. G.F. has received consulting fees from Bayer, Boehringer Ingelheim, NovoNordisk, and Servier; has received lecture fees from Novartis; has received trial committee membership fees from Impulse Dynamics, Vifor, and Medtronic; has served on trial committee for Cardior. S.D.A. reports grants from Abbott Vascular and Vifor (International) Ltd, consulting fees from Abbott Vascular, Bayer, Brahms GmbH, Cardiac Dimensions, Cordio, Novartis, Servier, and Vifor (International) Ltd. F.Z. reports payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from Boehringer Ingelheim, Amgen, CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno Pharmaceutical, Applied Therapeutics, Merck, and Bayer; other financial or non-financial interests in CVCT and Cardiorenal, and is a Trial Executive Committee member of the Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance (trial sponsor). All other authors have nothing to disclose.

References

1. van der Pol A, Hoes MF, de Boer RA, van der Meer P. Cardiac foetal reprogramming: A tool to exploit novel treatment targets for the failing heart. *J Intern Med* 2020;**288**:491–506. https://doi.org/10.1111/joim.13094
2. Packer M. Foetal recapitulation of nutrient surplus signalling by O-GlcNAcylation and the failing heart. *Eur J Heart Fail* 2023;**25**:1199–1212. https://doi.org/10.1002/ejhf.2972
3. Dirkx E, da Costa Martins PA, De Windt LJ. Regulation of fetal gene expression in heart failure. *Biochim Biophys Acta* 2013;**1832**:2414–2424. https://doi.org/10.1016/j.bbadis.2013.07.023
4. Packer M. SGLT2 inhibitors: Role in protective reprogramming of cardiac nutrient transport and metabolism. *Nat Rev Cardiol* 2023;**20**:443–462. https://doi.org/10.1038/s41569-022-00824-4
5. Packer M. Mutual antagonism of hypoxia-inducible factor isoforms in cardiac, vascular, and renal disorders. *JACC Basic Transl Sci* 2020;**5**:961–968. https://doi.org/10.1016/j.jacbts.2020.05.006
6. Warbrick I, Rabkin SW. Hypoxia-inducible factor 1-alpha (HIF-1α) as a factor mediating the relationship between obesity and heart failure with preserved ejection fraction. *Obes Rev* 2019;**20**:701–712. https://doi.org/10.1111/obr.12828
7. Sant'Ana PG, Tomasi LC, Murata GM, Vileigas DF, Mota GAF, Souza SLB, *et al.* Hypoxia-inducible factor 1-alpha and glucose metabolism during cardiac remodeling progression from hypertrophy to heart failure. *Int J Mol Sci* 2023;**24**:6201. https://doi.org/10.3390/ijms24076201
8. Sato T, Ichise N, Kobayashi T, Fusagawa H, Yamazaki H, Kudo T, *et al.* Enhanced glucose metabolism through activation of HIF-1α covers the energy demand in a rat embryonic heart primordium after heartbeat initiation. *Sci Rep* 2022;**12**:74. https://doi.org/10.1038/s41598-021-03832-5
9. Krishnan J, Ahuja P, Bodenmann S, Knapik D, Perriard E, Krek W, *et al.* Essential role of developmentally activated hypoxia-inducible factor 1alpha for cardiac morphogenesis and function. *Circ Res* 2008;**103**:1139–1146. https://doi.org/10.1161/01.RES.0000338613.89841.c1
10. Kudová J, Procházková J, Vašiček O, Perečko T, Sedláčková M, Pešl M, *et al.* HIF-1alpha deficiency attenuates the cardiomyogenesis of mouse embryonic stem cells. *PLoS One* 2016;**11**:e0158358. https://doi.org/10.1371/journal.pone.0158358
11. Georgy M, Salhiyyah K, Yacoub MH, Chester AH. Role of hypoxia inducible factor HIF-1alpha in heart valves. *Glob Cardiol Sci Pract* 2023;e202309. https://doi.org/10.21542/gcsp.2023.9
12. Sui X, Wei H, Wang D. Novel mechanism of cardiac protection by valsartan: Synergetic roles of TGF-β1 and HIF-1α in Ang II-mediated fibrosis after myocardial infarction. *J Cell Mol Med* 2015;**19**:1773–1782. https://doi.org/10.1111/jcmm.12551
13. Feng W, Ying Z, Ke F, Mei-Lin X. Apigenin suppresses TGF-beta1-induced cardiac fibroblast differentiation and collagen synthesis through the downregulation of HIF-1alpha expression by miR-122-5p. *Phytomedicine* 2021;**83**:153481. https://doi.org/10.1016/j.phymed.2021.153481
14. Lei L, Mason S, Liu D, Huang Y, Marks C, Hickey R, *et al.* Hypoxia-inducible factor-dependent degeneration, failure, and malignant transformation of the heart in the absence of the von Hippel-Lindau protein. *Mol Cell Biol* 2008;**28**:3790–3803. https://doi.org/10.1128/MCB.01580-07
15. Watson CJ, Collier P, Tea I, Neary R, Watson JA, Robinson C, *et al.* Hypoxia-induced epigenetic modifications are associated with cardiac tissue fibrosis and the development of a myofibroblast-like phenotype. *Hum Mol Genet* 2014;**23**:2176–2188. https://doi.org/10.1093/hmg/ddt614
16. Han SS, Wang G, Jin Y, Ma ZL, Jia WJ, Wu X, *et al.* Investigating the mechanism of hyperglycemia-induced fetal cardiac hypertrophy. *PLoS One* 2015;**10**:e0139141. https://doi.org/10.1371/journal.pone.0139141
17. Zhang Y, Yan W, Ji X, Yue H, Li G, Sang N. Maternal NO2 exposure induces cardiac hypertrophy in male offspring via ROS-HIF-1α transcriptional regulation and aberrant DNA methylation modification of Csx/Nkx2.5. *Arch Toxicol* 2018;**92**:1563–1579. https://doi.org/10.1007/s00204-018-2166-3
18. Huang J, Elicker J, Bowens N, Liu X, Cheng L, Cappola TP, *et al.* Myocardin regulates BMP10 expression and is required for heart development. *J Clin Invest* 2012;**122**:3678–3691. https://doi.org/10.1172/JCI63635
19. Chen H, Shi S, Acosta L, Li W, Lu J, Bao S, *et al.* BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. *Development* 2004;**131**:2219–2231. https://doi.org/10.1242/dev.01094
20. Dang TTH, Yun JW. BMP10 positively regulates myogenic differentiation in C2C12 myoblasts via the Smad 1/5/8 signaling pathway. *Mol Cell Biochem* 2021;**476**:2085–2097. https://doi.org/10.1007/s11010-021-04064-x
21. Gu JN, Yang CX, Ding YY, Qiao Q, Di RM, Sun YM, *et al.* Identification of BMP10 as a novel gene contributing to dilated cardiomyopathy. *Diagnostics (Basel)* 2023;**13**:242. https://doi.org/10.3390/diagnostics13020242
22. Wang W, Niu Z, Wang Y, Li Y, Zou H, Yang L, *et al.* Comparative transcriptome analysis of atrial septal defect identifies dysregulated genes during heart septum morphogenesis. *Gene* 2016;**575**:303–312. https://doi.org/10.1016/j.gene.2015.09.016
23. Pashmforoush M, Lu JT, Chen H, Amand TS, Kondo R, Pradervand S, *et al.* Nkx2-5 pathways and congenital heart disease; loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block. *Cell* 2004;**117**:373–386. https://doi.org/10.1016/s0092-8674(04)00405-2
24. David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood* 2007;**109**:1953–1961. https://doi.org/10.1182/blood-2006-07-034124
25. Wang L, Rice M, Swist S, Kubin T, Wu F, Wang S, *et al.* BMP9 and BMP10 act directly on vascular smooth muscle cells for generation and maintenance of the contractile state. *Circulation* 2021;**143**:1394–1410. https://doi.org/10.1161/CIRCULATIONAHA.120.047375
26. Capasso TL, Li B, Volek HJ, Khalid W, Rochon ER, Anbalagan A, *et al.* BMP10-mediated ALK1 signaling is continuously required for vascular development and maintenance. *Angiogenesis* 2020;**23**:203–220. https://doi.org/10.1007/s10456-019-09701-0
27. Lamouille S, Mallet C, Feige JJ, Bailly S. Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis. *Blood* 2002;**100**:4495–4501. https://doi.org/10.1182/blood.V100.13.4495
28. Chen H, Brady Ridgway J, Sai T, Lai J, Warming S, Chen H, *et al.* Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development. *Proc Natl Acad Sci USA* 2013;**110**:11887–11892. https://doi.org/10.1073/pnas.1306074110

29. Kesherwani V, Shahshahan HR, Mishra PK. Cardiac transcriptome profiling of diabetic Akita mice using microarray and next generation sequencing. *PLoS One* 2017;**12**:e0182828. https://doi.org/10.1371/journal.pone.0182828
30. Sun S, Yang S, Zhang N, Yu C, Liu J, Feng W, *et al.* Astragalus polysaccharides alleviates cardiac hypertrophy in diabetic cardiomyopathy via inhibiting the BMP10-mediated signaling pathway. *Phytomedicine* 2023;**109**:154543. https://doi.org/10.1016/j.phymed.2022.154543
31. Laux DW, Young S, Donovan JP, Mansfield CJ, Upton PD, Roman BL. Circulating Bmp10 acts through endothelial Alk1 to mediate flow-dependent arterial quiescence. *Development* 2013;**140**:3403–3412. https://doi.org/10.1242/dev.095307
32. Peacock HM, Caolo V, Jones EA. Arteriovenous malformations in hereditary haemorrhagic telangiectasia: Looking beyond ALK1-NOTCH interactions. *Cardiovasc Res* 2016;**109**:196–203. https://doi.org/10.1093/cvr/cvv264
33. Qu X, Liu Y, Cao D, Chen J, Liu Z, Ji H, *et al.* BMP10 preserves cardiac function through its dual activation of SMAD-mediated and STAT3-mediated pathways. *J Biol Chem* 2019;**294**:19877–19888. https://doi.org/10.1074/jbc.RA119.010943
34. An P, Fan D, Guo Z, Liu FY, Li CF, Yang D, *et al.* Bone morphogenetic protein 10 alleviates doxorubicin-induced cardiac injury via signal transducer and activator of transcription 3 signaling pathway. *Bioengineered* 2022;**13**:7471–7484. https://doi.org/10.1080/21655979.2022.2048994
35. Sun L, Yu J, Qi S, Hao Y, Liu Y, Li Z. Bone morphogenetic protein-10 induces cardiomyocyte proliferation and improves cardiac function after myocardial infarction. *J Cell Biochem* 2014;**115**:1868–1876. https://doi.org/10.1002/jcb.24856
36. Morine KJ, Qiao X, Paruchuri V, Aronovitz MJ, Mackey EE, Buiten L, *et al.* Conditional knockout of activin like kinase-1 (ALK-1) leads to heart failure without maladaptive remodeling. *Heart Vessels* 2017;**32**:628–636. https://doi.org/10.1007/s00380-017-0955-x
37. Morine KJ, Qiao X, Paruchuri V, Aronovitz MJ, Mackey EE, Buiten L, *et al.* Reduced activin receptor-like kinase 1 activity promotes cardiac fibrosis in heart failure. *Cardiovasc Pathol* 2017;**31**:26–33. https://doi.org/10.1016/j.carpath.2017.07.004
38. Nakano N, Hori H, Abe M, Shibata H, Arimura T, Sasaoka T, *et al.* Interaction of BMP10 with Tcap may modulate the course of hypertensive cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* 2007;**293**:H3396–H3403. https://doi.org/10.1152/ajpheart.00311.2007
39. Tang N, Rao S, Ying Y, Huang Y. New insights into BMP9 signaling in organ fibrosis. *Eur J Pharmacol* 2020;**882**:173291. https://doi.org/10.1016/j.ejphar.2020.173291
40. Scharpfenecker M, Floot B, Korlaar R, Russell NS, Stewart FA. ALK1 heterozygosity delays development of late normal tissue damage in the irradiated mouse kidney. *Radiother Oncol* 2011;**99**:349–355. https://doi.org/10.1016/j.radonc.2011.05.061
41. Winters J, Kawczynski MJ, Gilbers MD, Isaacs A, Zeemering S, Bidar E, *et al.* Circulating BMP10 levels associate with late postoperative atrial fibrillation and left atrial endomysial fibrosis. *JACC Clin Electrophysiol* 2024;**10**:1326–1340. https://doi.org/10.1016/j.jacep.2024.03.003
42. Hennings E, Blum S, Aeschbacher S, Coslovsky M, Knecht S, Eken C, *et al.*; Swiss-AF Investigators. Bone morphogenetic protein 10-a novel biomarker to predict adverse outcomes in patients with atrial fibrillation. *J Am Heart Assoc* 2023;**12**:e028255. https://doi.org/10.1161/JAHA.122.028255
43. Hijazi Z, Benz AP, Lindbäck J, Alexander JH, Connolly SJ, Eikelboom JW, *et al.* Bone morphogenetic protein 10: A novel risk marker of ischaemic stroke in patients with atrial fibrillation. *Eur Heart J* 2023;**44**:208–218. https://doi.org/10.1093/eurheartj/ehac632
44. Ceelen DCH, Bracun V, van Essen BJ, Voors AA, de Boer RA, Ter Maaten JM, *et al.* Circulating bone morphogenetic protein 10 as a novel marker of atrial stress and remodelling in heart failure. *Heart* 2025;**111**:172–179. https://doi.org/10.1136/heartjnl-2024-324486
45. Alt A, Miguel-Romero L, Donderis J, Aristorena M, Blanco FJ, Round A, *et al.* Structural and functional insights into endoglin ligand recognition and binding. *PLoS One* 2012;**7**:e29948. https://doi.org/10.1371/journal.pone.0029948
46. Mercado-Pimentel ME, Hubbard AD, Runyan RB. Endoglin and Alk5 regulate epithelial-mesenchymal transformation during cardiac valve formation. *Dev Biol* 2007;**304**:420–432. https://doi.org/10.1016/j.ydbio.2006.12.038
47. Singh E, Phillips HM, Arthur HM. Dynamic changes in endoglin expression in the developing mouse heart. *Gene Expr Patterns* 2021;**39**:119165. https://doi.org/10.1016/j.gep.2020.119165
48. Banerjee S, Dhara SK, Bacanamwo M. Endoglin is a novel endothelial cell specification gene. *Stem Cell Res* 2012;**8**:85–96. https://doi.org/10.1016/j.scr.2011.08.006
49. López-Novoa JM, Bernabeu C. The physiological role of endoglin in the cardiovascular system. *Am J Physiol Heart Circ Physiol* 2010;**299**:H959–H974. https://doi.org/10.1152/ajpheart.01251.2009
50. Ikemoto T, Hojo Y, Kondo H, Takahashi N, Hirose M, Nishimura Y, *et al.* Plasma endoglin as a marker to predict cardiovascular events in patients with chronic coronary artery diseases. *Heart Vessels* 2012;**27**:344–351. https://doi.org/10.1007/s00380-011-0163-z
51. Pomeraniec L, Hector-Greene M, Ehrlich M, Blobe GC, Henis YI. Regulation of TGF-β receptor hetero-oligomerization and signaling by endoglin. *Mol Biol Cell* 2015;**26**:3117–3127. https://doi.org/10.1091/mbc.E15-02-0069
52. Hiepen C, Mendez P-L, Knaus P. It takes two to tango: Endothelial TGFβ/BMP signaling crosstalk with mechanobiology. *Cells* 2020;**9**:1965. https://doi.org/10.3390/cells9091965
53. Shyu KG. The role of endoglin in myocardial fibrosis. *Acta Cardiol Sin* 2017;**33**:461–467. https://doi.org/10.6515/acs20170221b
54. Varejckova M, Gallardo-Vara E, Vicen M, Vitverova B, Fikrova P, Dolezelova E, *et al.* Soluble endoglin modulates the pro-inflammatory mediators NF-κB and IL-6 in cultured human endothelial cells. *Life Sci* 2017;**175**:52–60. https://doi.org/10.1016/j.lfs.2017.03.014
55. Yeh TC, Wu YC, Wong TY, Sun GC, Tseng CJ, Cheng PW. Dapagliflozin prevents ERK activation and SGLT2-dependent endoglin upregulation in a mechanically provoked cardiac injury model. *Physiol Rep* 2024;**12**:e15990. https://doi.org/10.14814/phy2.15990
56. Kapur NK, Wilson S, Yunis AA, Qiao X, Mackey E, Paruchuri V, *et al.* Reduced endoglin activity limits cardiac fibrosis and improves survival in heart failure. *Circulation* 2012;**125**:2728–2738. https://doi.org/10.1161/CIRCULATIONAHA.111.080002
57. Kapur NK, Qiao X, Paruchuri V, Mackey EE, Daly GH, Ughreja K, *et al.* Reducing endoglin activity limits calcineurin and TRPC-6 expression and improves survival in a mouse model of right ventricular pressure overload. *J Am Heart Assoc* 2014;**3**:e000965. https://doi.org/10.1161/JAHA.114.000965
58. Kapur NK, Heffernan KS, Yunis AA, Parpos P, Kiernan MS, Sahasrabudhe NA, *et al.* Usefulness of soluble endoglin as a noninvasive measure of left ventricular filling pressure in heart failure. *Am J Cardiol* 2010;**106**:1770–1776. https://doi.org/10.1016/j.amjcard.2010.08.018
59. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, *et al.*; EMPEROR-Reduced Trial Investigators. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med* 2020;**383**:1413–1424. https://doi.org/10.1056/NEJMoa2022190
60. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, *et al.*; EMPEROR-Preserved Trial Investigators. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med* 2021;**385**:1451–1461. https://doi.org/10.1056/NEJMoa2107038
61. Teichmann U, Kessel M. Highly restricted BMP10 expression in the trabeculating myocardium of the chick embryo. *Dev Genes Evol* 2004;**214**:96–98. https://doi.org/10.1007/s00427-003-0380-2
62. Kahr PC, Piccini I, Fabritz L, Greber B, Schöler H, Scheld HH, *et al.* Systematic analysis of gene expression differences between left and right atria in different mouse strains and in human atrial tissue. *PLoS One* 2011;**6**:e26389. https://doi.org/10.1371/journal.pone.0026389
63. Tessari A, Pietrobon M, Notte A, Cifelli G, Gage PJ, Schneider MD, *et al.* Myocardial Pitx2 differentially regulates the left atrial identity and ventricular asymmetric remodeling programs. *Circ Res* 2008;**102**:813–822. https://doi.org/10.1161/CIRCRESAHA.107.163188
64. Liu L, Liang Y, Lan QG, Chen JZ, Wang R, Zhao JH, *et al.* Bone morphogenetic protein 10 and atrial fibrillation. *Int J Cardiol Heart Vasc* 2024;**51**:101376. https://doi.org/10.1016/j.ijcha.2024.101376
65. Chua W, Cardoso VR, Guasch E, Sinner MF, Al-Taie C, Brady P, *et al.* An angiopoietin 2, FGF23, and BMP10 biomarker signature differentiates atrial fibrillation from other concomitant cardiovascular conditions. *Sci Rep* 2023;**13**:16743. https://doi.org/10.1038/s41598-023-42331-7
66. Meyre PB, Aeschbacher S, Blum S, Voellmin G, Kastner PM, Hennings E, *et al.* Biomarkers associated with rhythm status after cardioversion in patients with atrial fibrillation. *Sci Rep* 2022;**12**:1680. https://doi.org/10.1038/s41598-022-05769-9
67. Reyat JS, Chua W, Cardoso VR, Witten A, Kastner PM, Kabir SN, *et al.* Reduced left atrial cardiomyocyte PITX2 and elevated circulating BMP10 predict atrial fibrillation after ablation. *JCI Insight* 2020;**5**:e139179. https://doi.org/10.1172/jci.insight.139179
68. Gkarmiris KI, Lindbäck J, Alexander JH, Granger CB, Kastner P, Lopes RD, *et al.* Repeated measurement of the novel atrial biomarker BMP10 (bone morphogenetic protein 10) refines risk stratification in anticoagulated patients with atrial fibrillation: Insights from the ARISTOTLE trial. *J Am Heart Assoc* 2024;**13**:e033720. https://doi.org/10.1161/JAHA.123.033720
69. Hennings E, Aeschbacher S, Coslovsky M, Paladini RE, Meyre PB, Voellmin G, *et al.* Association of bone morphogenetic protein 10 and recurrent atrial fibrillation after catheter ablation. *Europace* 2023;**25**:euad149. https://doi.org/10.1093/europace/euad149
70. Chua W, Khashaba A, Canagarajah H, Nielsen JC, di Biase L, Haeusler KG, *et al.* Disturbed atrial metabolism, shear stress, and cardiac load contribute to atrial fibrillation after ablation: AXAFA biomolecule study. *Europace* 2024;**26**:euae028. https://doi.org/10.1093/europace/euae028

71. Castoldi G, Carletti R, Ippolito S, Colzani M, Barzaghi F, Stella A, *et al.* Renal anti-fibrotic effect of sodium glucose cotransporter 2 inhibition in angiotensin ii-dependent hypertension. *Am J Nephrol* 2020;**51**:119–129. https://doi.org/10.1159/000505144
72. Wang J, Lv X, A-Ni-Wan AS, Tian SS, Wang JM, Liu HY, *et al.* Canagliflozin alleviates high glucose-induced peritoneal fibrosis via HIF-1α inhibition. *Front Pharmacol* 2023;**14**:1152611. https://doi.org/10.3389/fphar.2023.1152611
73. Bessho R, Takiyama Y, Takiyama T, Kitsunai H, Takeda Y, Sakagami H, *et al.* Hypoxia-inducible factor-1α is the therapeutic target of the SGLT2 inhibitor for diabetic nephropathy. *Sci Rep* 2019;**9**:14754. https://doi.org/10.1038/s41598-019-51343-1
74. Finnson KW, Philip A. Endoglin in liver fibrosis. *J Cell Commun Signal* 2012;**6**:1–4. https://doi.org/10.1007/s12079-011-0154-y
75. Huang Q, Xiao R, Lu J, Zhang Y, Xu L, Gao J, *et al.* Endoglin aggravates peritoneal fibrosis by regulating the activation of TGF-β/ALK/Smads signaling. *Front Pharmacol* 2022;**13**:973182. https://doi.org/10.3389/fphar.2022.973182
76. Chen X, Yang Q, Bai W, Yao W, Liu L, Xing Y, *et al.* Dapagliflozin attenuates myocardial fibrosis by inhibiting the TGF-β1/Smad signaling pathway in a normoglycemic rabbit model of chronic heart failure. *Front Pharmacol* 2022;**13**:873108. https://doi.org/10.3389/fphar.2022.873108
77. Zhang Y, Lin X, Chu Y, Chen X, Du H, Zhang H, *et al.* Dapagliflozin: A sodium-glucose cotransporter 2 inhibitor, attenuates angiotensin II-induced cardiac fibrotic remodeling by regulating TGFβ1/Smad signaling. *Cardiovasc Diabetol* 2021;**20**:121. https://doi.org/10.1186/s12933-021-01312-8
78. Tian J, Zhang M, Suo M, Liu D, Wang X, Liu M, *et al.* Dapagliflozin alleviates cardiac fibrosis through suppressing EndMT and fibroblast activation via AMPKα/TGF-β/Smad signalling in type 2 diabetic rats. *J Cell Mol Med* 2021;**25**:7642–7659. https://doi.org/10.1111/jcmm.16601
79. Hanna A, Humeres C, Frangogiannis NG. The role of Smad signaling cascades in cardiac fibrosis. *Cell Signal* 2021;**77**:109826. https://doi.org/10.1016/j.cellsig.2020.109826
80. Chen K, Mehta JL, Li D, Joseph L, Joseph J. Transforming growth factor beta receptor endoglin is expressed in cardiac fibroblasts and modulates profibrogenic actions of angiotensin II. *Circ Res* 2004;**95**:1167–1173. https://doi.org/10.1161/01.RES.0000150369.68826.2f