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RESEARCH ARTICLE

Circulating dipeptidyl peptidase 3 is a myocardial depressant factor: dipeptidyl peptidase 3 inhibition rapidly and sustainably improves haemodynamics

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Aims Acute heart failure is a high mortality disease and its pathophysiology is not completely understood. Dipeptidyl peptidase 3 (DPP3) is a cytosolic enzyme involved in angiotensin II and enkephalins cleavage. The aim of this study was to investigate the association of circulating DPP3 (cDPP3) levels and mortality in cardiogenic shock patients and to determine the effects of high cDPP3 on organ function in a heart failure (HF) model in mice.

Methods and results cDPP3 was measured in 174 patients in cardiogenic shock and high cDPP3 levels were associated with an increased short-term mortality risk (standardized hazard ratio: 1.4 (1.1–1.8)) and severe organ dysfunction. Additionally, a rapid decrease in cDPP3 in cardiogenic shock patients within 24 h of admission was associated with a favourable outcome. This study showed that injection of DPP3 induced myocardial depression (−10 ± 2% of shortening fraction) and impaired kidney haemodynamics (+0.30 ± 0.02 of renal resistive index) in healthy mice. cDPP3 inhibition by Procizumab, a specific antibody directed against cDPP3, promptly normalized cardiac function and kidney haemodynamics in an acute heart failure mouse model, with a marked reduction in oxidative stress and inflammatory signalling.

Conclusion Our study demonstrated cDPP3 is a newly discovered myocardial depressant factor, the levels of which at admission are associated with mortality in severe HF patients. Furthermore, inhibition of cDPP3 by Procizumab improved haemodynamics in a mouse model of HF. Our results suggest that DPP3 could be a new biomarker and biotarget for severe HF.

Keywords Acute heart failure • cDPP3 • Biomarkers • Immunotherapy • Procizumab • Antibody •

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Introduction

Acute heart failure (AHF) is a multifactorial systemic syndrome that is accompanied by cardiac injury. Heart failure (HF) triggers structural, neurohormonal, cellular, and molecular modifications that act together to mitigate HF and restore the heart's physiological function.[1–3] AHF is associated with a high in-hospital mortality rate ranging from 4% to 30%.[4] However, the pathophysiology of AHF remains poorly understood, particularly at the molecular level, and the therapeutic arsenal remains insufficient regardless of the aetiology of HF. Dipeptidyl peptidase 3 (DPP3) is a predominantly intracellular ubiquitous zinc-dependent metallopeptidase that is involved in the metabolism of peptides.[5] DPP3 has been implicated in blood pressure regulation,[6,7] inflammation,[6] and pain modulation[8] by inactivating bioactive peptides through cleavage, notably angiotensin II, enkephalins, and endorphins.[6] Nevertheless, the biological function of DPP3 is poorly understood.[6] Up to now only a few studies have investigated the role and possible clinical effects of circulating DPP3 (cDPP3). In an experimental model of angiotensin II-induced hypertension, Pang *et al.* demonstrated the *in vivo* normotensive role of intravenous injection of recombinant DPP3, consistent with the cleavage of angiotensin II by DPP3.[7] Recently, highly specific assays for the detection of cDPP3 were developed and an association between high levels of plasma cDPP3 activity and mortality in septic patients was reported.[9] The present study sought to clarify the association between cDPP3 levels and mortality in severe HF patients, and to determine the impact of cDPP3 on cardiac function and kidney haemodynamics, as a proof of concept for DPP3 inhibition as HF therapy.

Methods

Study population

The CardShock study (NCT01374867) has been described previously.[10] Blood samples were processed and stored at −80°C before transfer to the central laboratory for blinded DPP3 measurements [DPP3-luminometric immunoassay (DPP3-LIA), 4TEEN4 Pharmaceuticals GmbH, Hennigsdorf, Germany]; levels of cDPP3 in healthy humans are 13 ng/mL.[9] Routine laboratory analyses (i.e. PaO_2, lactate, etc.) were performed by the local laboratories of the study centres. The need for cardiovascular support was defined as the requirement for catecholamine during intensive care unit (ICU) hospitalization. Worsening of renal function was defined by an increase in renal Sequential Organ Failure Assessment (SOFA) score between baseline and 48 h.

Animal experimentation

Preclinical studies conformed to the National Institutes of Health *Guide for Care and Use of Laboratory Animals* and were approved by the Lariboisière–Villemin Animal Ethics Committee (04146.03). To respect the ethical Three Rs rules (3Rs), we used the minimum required number of animals for expecting a significant difference. Data collection was stopped in advance when final endpoints were obtained. Outliers, defined before the beginning of the study, were identified using Grubbs' test (extreme studentized deviate test); excluded outliers were not reported. (See Supplementary material online for details.)

Dipeptidyl peptidase 3 injections in healthy mice

Native human DPP3 (hDPP3) was purified from human blood cells as previously described[11] and injected into 3-month-old male C56Bl/6 mice (0.55 mg/kg). Of note, identity between human DPP3 (accession number NP_569710) and mouse DPP3 (accession number NP_001347640) is 93% and similarity is 96% over the entire length of the proteins (737 amino acids). hDPP3- and PBS-injected mice were monitored for 2 h by echocardiography and kidney ultrasound during the procedure before being sacrificed. To limit the impact of serial blood sampling on cardiac and renal function, the half-life of hDPP3 plasma concentrations was performed in different (satellite) mice by collecting 50 µL blood samples before, and 5 min, 15 min, 1 h, and 2 h after DPP3 injection. Two hours post-injection mice were sacrificed for terminal bleeding and the heart was collected for further analysis. DPP3-LIA was used to determine hDPP3 concentrations.[9]

We next spiked healthy human ($n = 2$) and mouse ($n = 2$) fresh heparin-plasma with increased concentrations of hDPP3 to reach concentrations found in the blood of patients with various acute diseases; angiotensins were measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS). (See Supplementary material online for details.)

Isoproterenol-induced heart failure model

Acute cardiac stress was provoked in 3-month-old C57Bl/6 male mice by subcutaneous injections of 300 mg/kg of isoproterenol (DL-isoproterenol hydrochloride, Sigma Chemical Co; ISO) in 0.9% NaCl (saline) twice daily for 2 days as previously described.[12] Sham mice ($n = 20$) only received saline. Procizumab (PCZ) (10 mg/kg i.v. $n = 14$) or phosphate-buffered saline (PBS) ($n = 21$) were intravenously injected in mice with isoproterenol-induced cardiac dysfunction assessed by a significant decrease in fractional shortening 12 h after the last isoproterenol injection. Mice were sacrificed 1, 6, or 24 h after injection. For the analysis of long-term effects, isoproterenol-induced HF (ISO-HF) + PBS ($n = 5$) and ISO-HF + PCZ ($n = 5$) mice were observed for 14 days. Heart and kidney function were monitored by echocardiography and kidney ultrasound, respectively. After the animals were sacrificed their organs were collected for analysis.

Procizumab injection in healthy mice

To assess possible effects of PCZ in mice without cardiac dysfunction, we injected the specific inhibitory antibody directly against cDPP3 in 3-month-old male C57Bl/6 male mice ($n = 3$, Sham + PCZ group). Heart function was monitored by echocardiography 1 and 6 h after PCZ injection.

Statistical analysis

CardShock study

Values are expressed as median and interquartile range (IQR), or as count and percentages, as appropriate. Group comparisons of continuous variables were performed using the Kruskal–Wallis test followed by Dunn's post hoc test. Categorical data were compared using Pearson's χ^2 test for count data. cDPP3, lactate, high-sensitivity troponin T (hsTnT), and creatinine data were log-transformed. Cox

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proportional-hazard regression was used to assess the association between cDPP3 and survival. For continuous variables, hazard ratios (HRs) were standardized to describe the HR for a biomarker change from quartile 1 to quartile 3. The concordance index (C index) is given as an effect measure. It is equivalent to the concept of area under the curve adopted for binary outcome. Kaplan–Meier plots were used for survival analysis. Correlation was calculated as the Spearman rank correlation (r). A two-sided P-value of 0.05 was considered for significance. The statistical analysis for CardShock was performed using R version 3.6.1 (http://www.r-project.org, library Design, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA).

Experimental studies

Data are presented as mean ± SD. For two-group comparisons, Wilcoxon signed-rank test or Wilcoxon rank-sum test were used as appropriate. Comparison between more than two groups was performed by repeated measures ANOVA or a Kruskal–Wallis test followed by Dunn's multiple comparison test, as appropriate. A P-value <0.05 was considered statistically significant. Statistical analysis for experimental studies was performed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Circulating dipeptidyl peptidase 3 plasma levels in cardiogenic shock patients

cDPP3 was measured in a cohort of 174 cardiogenic patients (CardShock study) with impaired myocardial function, for whom all-cause mortality follow-up data were available at 90 days.[10] Details of the clinical and the biological parameters of the study population are summarized in *Table 1*. At admission, median cDPP3 concentration was 33.4 ng/mL (IQR 20.0–65.3). Patients with cDPP3 levels above the median had higher lactate, creatinine, potassium, hsTnT, and lower estimated glomerular filtration rate (eGFR) and left ventricular ejection fraction than those with cDPP3 levels below the median (see *Table 1* for details). During the 90-day follow-up period, 72 (41%) patients died. Patients with cDPP3 values at admission greater than the median had higher mortality when compared with those with cDPP3 levels lower than the median (*Figure 1A*, $P = 0.0001$). Non-survivors had high cDPP3 levels at admission [42.9 ng/mL (IQR 28.4–86.6) vs. 26.5 ng/mL (IQR 17.1–54.9) in survivors, $P = 0.0002$] and at all other time points (Supplementary material online, *Figure S1A*). Consequently, cDPP3 levels at admission were predictive for 90-day mortality. Yet the C index of cDPP3 was 0.64 [95% confidence interval (CI) 0.57–0.70], $P = 0.0064$; of note, the C indices of other biomarkers at admission were NT-proBNP (0.61), C-reactive protein (0.51), hsTnT (0.57), lactate (0.72), and creatinine (0.66). Cox regression analysis showed an association between cDPP3 at admission and mortality with standardized HR 1.4 (1.1–1.8) (*Figure 1B*). Interestingly, some patients exhibited marked variations in cDPP3 levels during the first 24 h after admission (*Figure 1C*, $P < 0.0001$). Patients with a high concentration of cDPP3 at admission but a low concentration at 24 h (high to low group, HL) had a reduced 90-day mortality when compared with patients with high and sustained cDPP3 levels (high to high group, HH). Furthermore, decrease in cDPP3 concentration was associated with a decreased need for cardiovascular support and an improvement of renal function ($P < 0.0001$ and $P = 0.0056$, *Figure 1D* and *1E*). In contrast, patients with low cDPP3 at admission and increased values at 24 h (low to high group, LH) had a marked increase in mortality and greater need for organ support when compared with the patients with low and sustained cDPP3 levels (low to low group, LL; *Figure 1C* and *1D*). Of note, cDPP3 activity very strongly correlated with cDPP3 concentration in the plasma of all patients, regardless of the time point considered ($R^2 = 0.9964$, Supplementary material online, *Figure S1B*).

Circulating dipeptidyl peptidase 3 injection caused a deterioration in haemodynamics in healthy anaesthetized male mice

In order to assess the direct effects of cDPP3 on cardiovascular function and kidney haemodynamics, purified native human DPP3 (hDPP3)[11] was intravenously injected into healthy anaesthetized mice (*Figure 2A*). Injected hDPP3 had a half-life of about 20 min (*Figure 2B*), and hDPP3 concentrations returned to low values at 120 min, as previously observed in healthy rats.[9] Injection of hDPP3 provoked a rapid decrease in left ventricular shortening fraction (LVSF 61.6 ± 2.6% vs. 53.4 ± 2.0% at baseline, $P = 0.019$), which remained low for 10 min before progressively returning to baseline values at 120 min (*Figure 2C*). Individual values of shortening fraction between baseline and 15 min after hDPP3 injection are reported in the Supplementary material online, *Figure S2*. The renal resistive index (RRI), as an estimate of intrarenal haemodynamics, increased 60 min after hDPP3 injection (hDPP3: 0.77 ± 0.07 vs. Sham: 0.58 ± 0.06, $P = 0.027$, *Figure 2D*). LVSF and RRI remained stable in PBS-injected sham mice throughout the experiment. Although LVSF normalized after 120 min, dihydroethidium (DHE) labelling of the myocardium showed a higher reactive oxygen species production in the hearts of hDPP3-injected mice (4.8 ± 1.3 arbitrary units), when compared with those of sham animals (1.4 ± 0.7 arbitrary units, $P = 0.0286$, *Figure 2E* and *F*). Given the impact of DPP3 on angiotensin II degradation, we measured the angiotensin peptides by mass spectrometry fingerprinting. At baseline, mice had higher levels of angiotensin peptides when compared with humans (Supplementary material online, *Table S1*). Spiking human and mouse plasma samples with hDPP3 provoked a decrease of all angiotensins tested in both species, although the effect on angiotensin II was more pronounced in human samples (Supplementary material online, *Table S1*).

Dipeptidyl peptidase 3 pathway in isoproterenol-heart failure mouse model

The protocol for isoproterenol-induced HF (ISO-HF) is summarized in *Figure 3A*. Twelve hours after the last isoproterenol injection, ISO-HF mice exhibited signs of acute HF with: (i) a decrease in LVSF (47.3 ± 4.6% vs. 60.1 ± 3.8%, $P = 0.0012$, *Figure 3B*), (ii)

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Table 1 Patient characteristics from the CardShock study

Characteristics	All ($n = 174$)	DPP3, below median ($n = 88$)	DPP3, above median ($n = 86$)	*P*-value
DPP3 (pmol/L)	33.4 [20–65.3]	[0–33.4]	[33.4–886.5]	—
Age (years)	67 [60–75]	67 [61–77]	67[57–73]	0.3161
Women; *n*(%)	47 (27.0)	26 (29.5)	21 (24.4)	0.5547
BMI; mean (SD)	26.7 [24.2–29.4]	26.6 [24.2–29.07]	27.05 [24.2–29.7]	0.6386
Medical history, *n* (%)				
Coronary artery disease	51 (29.3)	24 (27.3)	27 (31.4)	0.6666
Previous myocardial infarction	45 (25.9)	21 (23.9)	24 (27.9)	0.6630
Previous PCI	29 (16.7)	13 (14.8)	16 (18.6)	0.6350
Previous CABG	11 (6.3)	6 (6.8)	5 (5.8)	0.9686
Heart failure	29 (16.7)	14 (15.9)	15 (17.4)	0.9459
IHD	59 (33.9)	26 (29.5)	33 (38.4)	0.2848
Hypertension	104 (59.8)	50 (56.8)	54 (62.8)	0.5166
Diabetes	52 (29.9)	28 (31.8)	24 (27.9)	0.6907
Asthma/COPD	19 (10.9)	10 (11.4)	9 (10.5)	0.9577
Renal insufficiency	22 (12.6)	9 (10.2)	13 (15.1)	0.4581
Stroke/TIA	16 (9.2)	10 (11.4)	6 (7)	0.4600
Smoker	70 (40.5)	36 (41.4)	34 (39.5)	0.9265
Clinical presentation				
Blood pressure (MAP; mmHg)	57 [50–63]	57 [52–61]	57 [48–66.5]	0.7125
Heart rate at enrolment (beats/min)	90 [70–110]	89 [71–110]	90 [69–110]	0.6999
Rhythm at enrolment, *n* (%)				
Sinus rhythm	125 (72.7)	68 (77.3)	57 (67.9)	0.2248
Atrial fibrillation. Other	26 (15.2)	13 (14.8)	13 (15.7)	0.9593
Baseline echocardiography				
LVEDD (mm)	51 [47–56]	51 [46–55]	52 [48.75–57.25]	0.1153
LVEF (%)	30 [20–42]	35 [20.75–45]	28 [20–35]	0.0196
Biochemistry				
Hb (g/dL)	13.1 [11.2–14.4]	13.2 [11.1–14.5]	13.0 [11.5–14.1]	0.9244
Sodium (mmol/L)	137 [134–140]	138 [134–140]	136 [133–139]	0.1638
Potassium (mmol/L)	4.1 [3.8–4.6]	3.9 [3.7–4.24]	4.3 [3.83–5]	0.0010
Lactate (mmol/L)	2.8 [1.8–5.8]	2.3 [1.4–3.3]	4 [2.3–7.5]	<0.0001
pH	7.30 [7.22–7.40]	7.33 [7.24–7.4]	7.3 [7.21–7.4]	0.4185
hsTnT (ng/mL)	2120 [386–5313]	1166 [285–3795]	2750 [976–8716]	0.0028
NT-proBNP (pg/mL)	2782 [631–9657]	2067 [676–8299]	3706 [590–11 623]	0.2286
Creatinaemia (μmol/L)	103 [78–141]	97 [80–123]	115 [89–168]	0.0069
eGFR (mL/min)	61 [40–86]	67.2 [43.5–89.3]	54.9 [32.2–75.4]	0.0119
CRP (mg/L)	17 [4–53]	13 [3–43]	22.4 [1–97]	0.4476
Use of vasoactive medication during the first 96 h from detection of shock, *n* (%)				
Epinephrine	28 (17.1)	6 (7)	22 (28.2)	0.0007
Norepinephrine	129 (75.4)	66 (75.9)	63 (75)	0.9627
Dopamine	29 (17.2)	10 (11.6)	19 (22.9)	0.0823
Dobutamine	92 (54.4)	39 (45.3)	53 (63.9)	0.0238
Levosimendan	46 (27.1)	24 (27.9)	22 (26.2)	0.9369
Management and outcomes, *n* (%)				
Trombolysis	16 (9.4)	8 (9.3)	8 (9.4)	0.8119
Angiogram	130 (90.9)	66 (86.8)	64 (95.5)	0.1310
PCI	117 (67.6)	59 (67.8)	58 (67.4)	0.9125
Mechanical haemodynamics support				
IABP	96 (55.2)	45 (51.1)	51 (59.3)	0.3521
LVAD	6 (3.5)	2 (2.3)	4 (4.8)	0.6459
ECMO	3 (1.8)	0 (0)	3 (3.6)	0.2276
Admission ICU/CCU	63 (36.2)	33 (37.5)	30 (34.9)	0.8405
RRT	19 (11.2)	8 (9.3)	11 (13.1)	0.5883

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Table 1 Continued

Characteristics	All ($n = 174$)	DPP3, below median ($n = 88$)	DPP3, above median ($n = 86$)	*P*-value
Surgery				
CABG	6 (3.5)	3 (3.4)	3 (3.6)	0.7100
Valvular surgery	7 (4.1)	4 (4.6)	3 (3.6)	0.9622
Other major surgery	14 (8.2)	7 (8.1)	7 (8.3)	0.8157
90-day mortality	72 (41.4)	23 (26.1)	49 (57)	0.0001

Values are expressed as median and interquartile range (IQR), or as count and percentages, as appropriate. Group comparisons of continuous variables were performed using the Kruskal–Wallis test. Categorical data were compared using Pearson's χ^2 test. Circulating dipeptidyl peptidase 3 (cDPP3), lactate, high-sensitivity troponin T (hsTnT), and creatinine data were log-transformed. All statistical tests were two-tailed and a two-sided *P*-value of 0.05 was considered for significance.
BMI, body mass index; CABG, coronary artery bypass grafting; CCU, critical care unit; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ECMO, extracorporeal membrane oxygenation; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; hsTnT, high-sensitivity troponin T; IABP, intra-aortic balloon pump; ICU, intensive care unit; IHD, ischaemic heart disease; LVAD, left ventricular assist device; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; NT-proBNP, N-terminal pro brain natriuretic peptide; PCI, percutaneous coronary intervention; RRT, renal replacement therapy; TIA, transient ischaemic attack.

a decrease in cardiac output (CO) (13.11 ± 2.15 mL/min vs. 22.65 ± 6.58 mL/min, $P = 0.0177$, Supplementary material online, *Figure S3A*), (iii) lung congestion (lung weight: 9.85 ± 1.32 mg/mm tibia length vs. 7.96 ± 1.02 mg/mm tibia length, $P = 0.0293$, Supplementary material online, *Figure S3B*), (iv) an increase in E/A wave mitral ratio (1.46 ± 0.19 vs. 1.23 ± 0.12, $P = 0.0303$, Supplementary material online, *Figure S3C*), and (v) an increase in RRI (0.73 ± 0.14 vs. 0.53 ± 0.09, $P = 0.0183$, *Figure 3C*). Importantly, cDPP3 activity was markedly increased in the plasma of ISO-HF mice when compared with sham animals (51.6 ± 19 U/L vs. 22.1 ± 5.1 U/L, $P = 0.0423$, *Figure 3D*). Furthermore, cardiac DPP3 mRNA and protein levels were higher in ISO-HF mice when compared with sham mice (0.96 ± 0.16 arbitrary units vs. 0.62 ± 0.17 arbitrary units, $P = 0.0159$ and 1.16 ± 0.65 arbitrary units vs. 0.69 ± 0.65 arbitrary units, $P = 0.0317$, respectively; Supplementary material online, *Figure S3D* and *S3E*).

Benefits of procizumab injection in heart failure model in mice

PCZ injection rapidly normalized the LVSF of ISO-HF + PCZ mice within 1 h after injection when compared with ISO-HF + PBS group ($60.7 \pm 4.36\%$ vs. $48.1 \pm 3.96\%$, $P = 3.32 \times 10^{-7}$, *Figure 3E*); LVSF remained normal at 24 h (*Figure 3E*) and 14 days after the single initial PCZ injection in ISO-HF + PCZ mice (Supplementary material online, *Figure S3F*). PCZ also improved stroke volume and CO in ISO-HF + PCZ mice within 6 h (*Figure 3F*). The injection of a non-active IgG had no effect on LVSF in ISO-HF mice and values were similar to those of the ISO-HF + PBS at day 8 and day 14 (Supplementary material online, *Figure S3F*). Supplementary material online *Figure S4A* shows individual values of shortening fraction before and 1 h after PCZ injection in the ISO-HF + PCZ group. Concerning renal haemodynamics, RRI decreased towards normal values at 6 and 24 h in ISO-HF + PCZ animals whereas it remained unchanged in ISO-HF + PBS mice (0.66 ± 0.14 vs. 0.81 ± 0.15, $P = 0.073$, *Figure 3G*). RRI remained stable throughout the experiment in sham mice. In addition to the improvement in haemodynamics, PCZ rapidly reduced oxidative stress in the heart of ISO-HF + PCZ mice when compared with those of ISO-HF + PBS animals (2.61 ± 1.02 AU vs. 9.30 ± 1.88 AU at 1 h, $P = 0.0286$) (*Figure 3H* and *3I*). LVSF was not modified in the Sham + PCZ group compared with Sham + PBS, 1 h and 6 h after PCZ injection ($P = 0.9554$, Supplementary material online, *Figure S4B*). Supplementary material online *Figure S4C* shows exemplary pictures of echocardiography and renal Doppler in the ISO-HF + PCZ group (baseline, day 3 and 24 h after PCZ injection).

Discussion

Our study demonstrated that DPP3, a cytosolic enzyme, is a newly myocardial depressant factor, the plasma levels of which at admission are associated with mortality in patients with cardiogenic shock. Additionally, in preclinical studies, excess cDPP3 provoked cardiac depression, associated with alteration in inflammation pathway and angiotensin peptide concentrations, while cDPP3 inhibition improves cardiac contraction in a mouse model of HF.

The ISO-HF mouse model was chosen to better mimic the pathophysiology of acute HF and evaluate the impact of cDPP3 blockade on cardiac function and kidney haemodynamics. This model exhibits cardiac dysfunction and diffuse cellular necrosis[13–15] as observed during cardiogenic shock.[16] Since cDPP3 is elevated in severe HF and in the ISO-HF model, specific inhibition of cDPP3 was tested *in vitro* by various mouse monoclonal antibodies raised against the protein, resulting in the selection of PCZ, which was later humanized (Supplementary material online, *Figure S5*). Crystal structures of hDPP3 in apo and substrate-bound forms show that the enzyme undergoes a large conformational change between the open (apo) state and the closed (substrate-bound state).[17] PCZ binds to a conserved loop in the vicinity of the active site that remains accessible in both the open and closed states, therefore likely precluding the transition between cDPP3 catalytic states and therefore inhibiting its enzymatic activity.

Using PCZ, our study demonstrated the key role of cDPP3 in altered haemodynamics in acute HF in mice. Indeed, cDPP3 inhibition by PCZ was associated with improved cardiac contraction and kidney haemodynamics. In the present study, the

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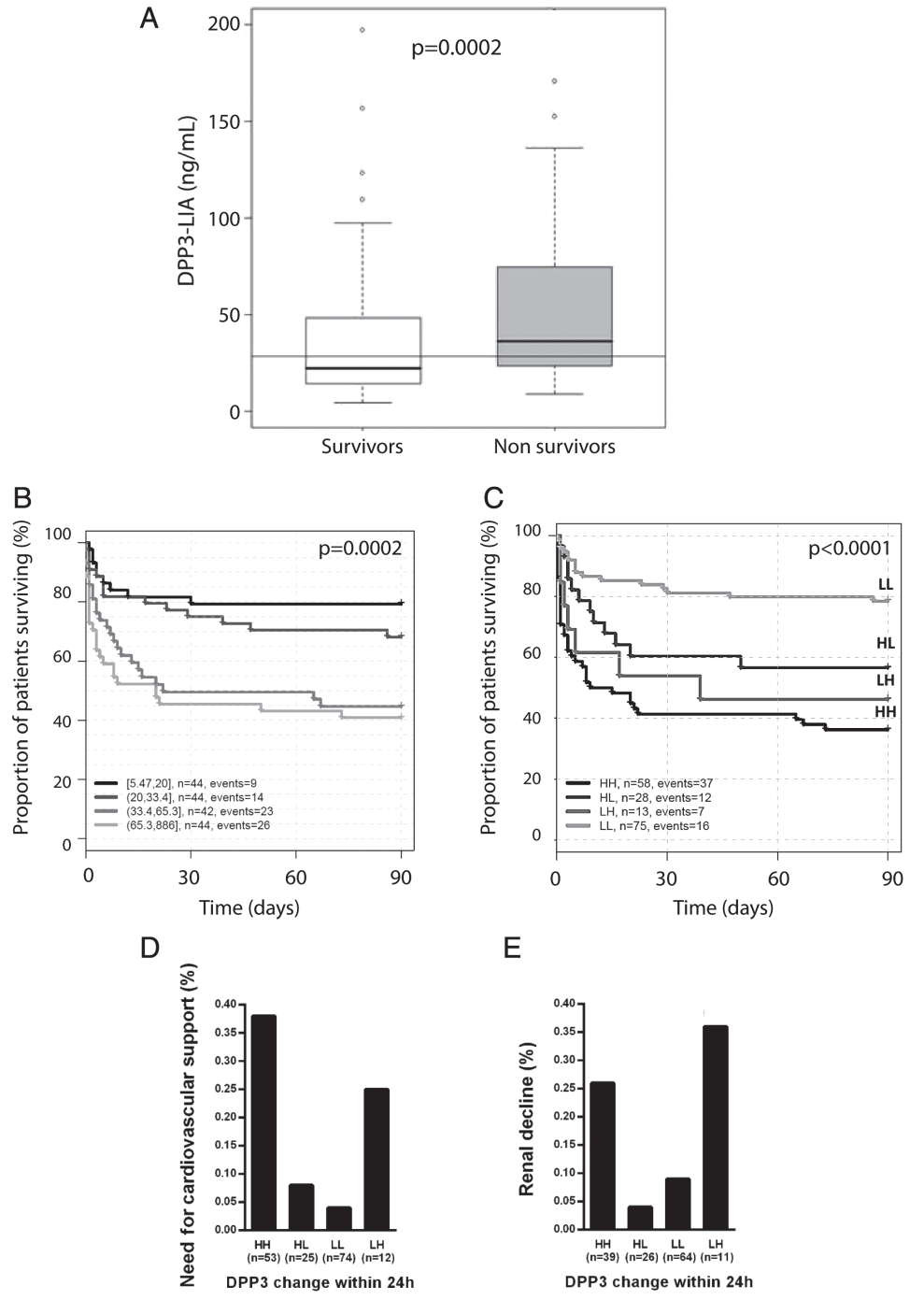

Figure 1 High circulating dipeptidyl peptidase 3 (cDPP3) plasma levels correlate with high risk of organ dysfunction and mortality in cardiogenic shock patients. (*A*) cDPP3 concentrations in survivors (white) and non-survivors (grey) at baseline. Comparison was made by Mann–Whitney rank-sum test. The outliers on the graph were included in the analysis. Kaplan–Meier plot of the cDPP3 levels at admission by quartiles (*B*), and combination at admission and 24 h cDPP3 (*C*). Cut-off for both time points is the median of admission cDPP3 (33.4 ng/mL). The value of 33.4 ng/mL of cDPP3 was used at baseline and 24 h. HH: cDPP3 levels above the median at admission and 24 h; HL: cDPP3 levels above the median at admission and the below median at 24 h; LL: cDPP3 levels below the median at admission and 24 h; LH: cDPP3 levels below the median on admission but above median at 24 h. (*D*) Proportion of patients in need of cardiovascular support within the first 96 h after admission (available in 164 patients), according to the HH, HL, LL, and LH groups. (*E*) Proportion of patients with renal decline, defined as an increase in renal sequential organ failure assessment (SOFA) score between baseline and 48 h (available in 140 patients), according to the HH, HL, LL, and LH groups.

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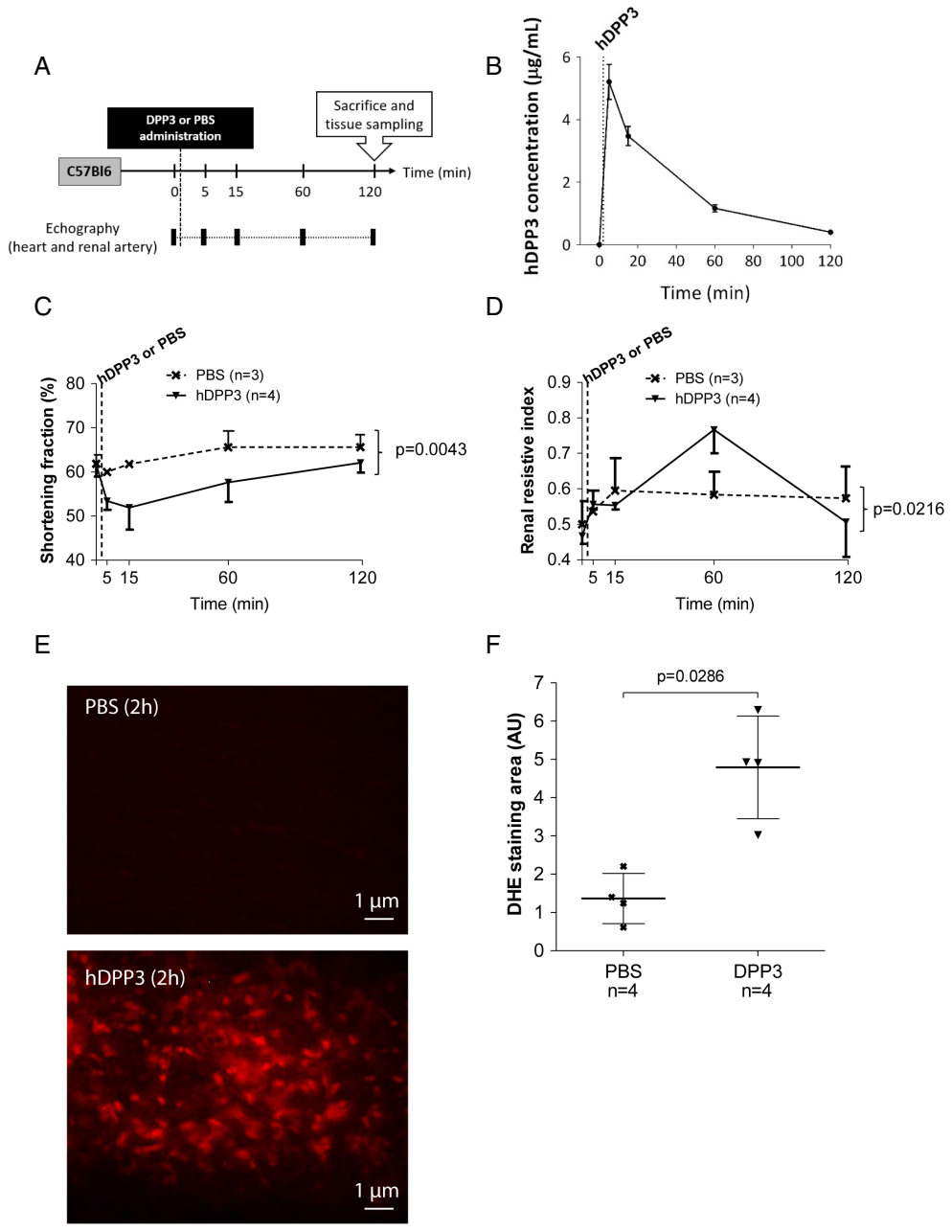

Figure 2 Effects of dipeptidyl peptidase 3 (DPP3) injection on heart function and kidney haemodynamics in healthy mice. (*A*) Schematic representation of the protocol used for human DPP3 (hDPP3) injections in mice (0.55 mg/kg). (*B*) hDPP3 plasma concentrations in satellite mice. (*C*) Left ventricular shortening fraction of healthy mice injected or not with hDPP3. (*D*) Renal resistive index of healthy mice injected or not with DPP3. (*E*) Dihydroethidium (DHE) staining of myocardial cross-sections. (*F*) Relative quantification of DHE staining of myocardial slices. Comparison was made by Mann–Whitney rank-sum test. PBS, phosphate-buffered saline.

benefits of PCZ are in line with detrimental effects seen with hDPP3 intravenous injection both herein and in a previous study showing that cDPP3 reduced blood pressure.[7] In the CardShock study, the striking detrimental association between high cDPP3, related to cellular necrosis from the heart and/or from other injured organs, and survival suggests that the negative effects of high cDDP3 in our patients might include worsening in both haemodynamics and tissue injury. This is supported by the great need for cardiovascular support and/or the marked renal decline seen in our Cardiogenic patients patients with persistent high cDPP3. Our clinical data are in agreement with results from the OptimaCC study[18] demonstrating that high levels of cDPP3 levels in cardiogenic shock patients, after acute myocardial infarction, are associated with low cardiac index and high occurrence of

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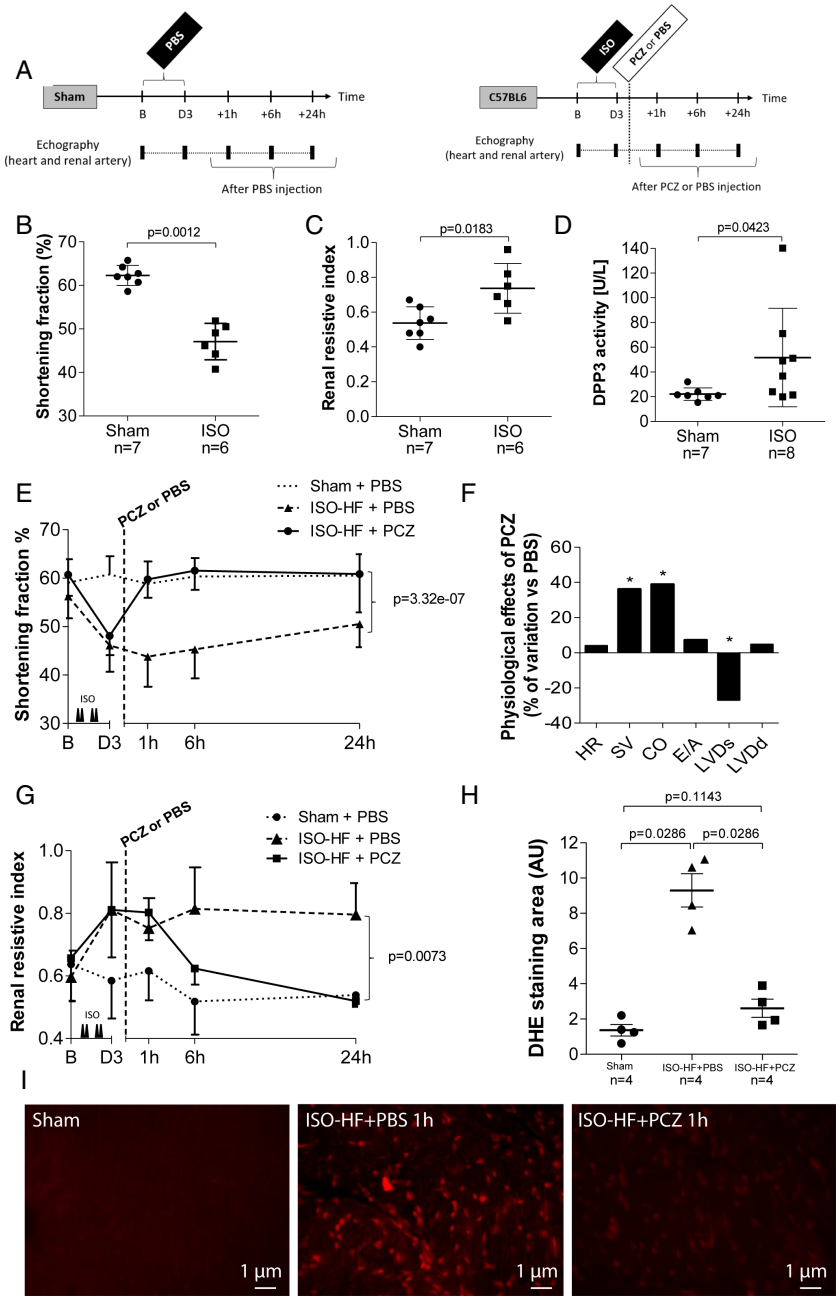

Figure 3 Procizumab rapidly and sustainably improves haemodynamics in isoproterenol-injected (ISO) mice. (*A*) Schematic representation of the protocol used to provoke heart failure in mice. (*B*) Left ventricular shortening fraction of sham and ISO mice at day 3, i.e. 12 h after the last isoproterenol injection. Comparison was made by Mann–Whitney rank-sum test. (*C*) Renal resistivity of sham and ISO mice at day 3. Comparison was made by Mann–Whitney rank-sum test. (*D*) Plasma mouse circulating dipeptidyl peptidase 3 (DPP3) activity in sham and ISO mice. (*E*) Left ventricular shortening fraction in sham + phosphate-buffered asaline (PBS) ($n = 5$), isoproterenol-induced heart failure (ISO-HF) + PBS ($n = 5$) and ISO-HF + procizumab (PCZ) ($n = 5$) mice until 24 h after treatment. Comparison was made by repeated measures ANOVA. (*F*) Difference between ISO-HF + PBS and ISO-HF + PCZ groups in haemodynamics 6 h after PCZ or PBS injection. (*$P < 0.05$ between ISO + PBS and ISO + PCZ groups). (*G*) Renal resistive index in sham + PBS ($n = 5$), ISO-HF + PBS ($n = 5$) and ISO-HF + PCZ ($n = 5$) mice 24 h after PCZ or PBS injection. Comparison was made by repeated measures ANOVA. (*H*) Quantification of myocardial dihydroethidium (DHE) labelling. Comparison was made by Mann–Whitney rank-sum test. (*I*) DHE labelling of cardiomyocytes from sham and ISO-HF + PBS and ISO-HF + PCZ mice. CO, cardiac output; E/A, mitral Doppler wave; HR, heart rate; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; SV, stroke volume.

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refractory shock and impaired kidney function. Accordingly, our data support the need to assess the benefits of PCZ to restore haemodynamics and to improve outcome in acute HF patients.

Our data provide solid evidence of a central role for DPP3 in the pathophysiology of cardiac dysfunction. Mechanisms of DPP3-induced myocardial depression are unknown and need further exploration. Our work shows that both the DPP3 depressant myocardial effects and the benefits of its inhibition by PCZ on contractile function are rapid. An alternative explanation is the rapid effect of DPP3 on the metabolism of cardiovascular peptides with known cardiovascular properties. The present study shows incremental and striking, especially in humans, reduction of angiotensins with increasing concentration of DPP3. Further studies should assess whether circulating enkephalins, another well-known substrate of DPP3 with positive inotropic properties,[19,20] are affected by excess cDPP3. Future studies also need to address these cardiovascular mediators *in vivo* during DPP3 or PCZ infusion.

Our study has some limitations. First, we studied the effect of PCZ in a unique model of HF. However, the combination of both human and preclinical study was designed to provide a proof of concept of cDPP3 as a possible biomarker and therapeutic target in HF. Our results would need confirmation in other preclinical models of HF,[9] especially in sepsis that combines cardiovascular dysfunction and excess inflammation, which is beyond the proof of concept design of this study. Second, the number of tested animals was rather low. However, with respect to the main results section, individual data showed that cDPP3 (reduction in shortening fraction) and PCZ (improvement in shortening fraction) effects were similar in all tested animals. Although performed in the plasma of two mice, *in vitro* experiments showed that high concentrations of DPP3 led to a reduction of plasma concentration of all angiotensin peptides. Additionally, a similar effect was observed in human plasma. Our preclinical data are a proof of concept study and the results require confirmation in large animal models. In addition, cardiac and vascular haemodynamic measurements and further biological analyses should be performed in future preclinical studies to better understand the mechanism(s) of action of PCZ. Thirdly, the biological effects of cDPP3 inhibition by PCZ are not completely understood and deserve more detailed analyses. In particular, measurements of mediators of the renin–angiotensin–aldosterone system should be performed in a large number of animals and humans in the presence or absence of PCZ and of angiotensin II receptor blockers. Lastly, prognostic properties of cDPP3 in CS need confirmation in large cohorts in order to assess added values compared with previously described clinical and biological severity scores.

In conclusion, our study suggests that cDPP3, when elevated in the plasma, could be a potential myocardial depressant factor associated with high mortality and organ dysfunction in severe HF patients and in a mouse model of HF. Future studies should compare the prognostic properties of cDPP3 and other biomarkers usually used in severe and/or advanced HF, namely troponin or natriuretic peptides. Our study further showed that inhibition of cDPP3 by PCZ promptly and sustainably restored cardiac contraction in HF in mice. Mechanisms of PCZ action and confirmation of its favourable effects on haemodynamics in large animal models and in humans are needed in order to propose it as a novel therapy in severe heart failure.

Supplementary Information

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

Figure S1. ISO, isoproterenol; Analysis of cDPP3 concentrations at admission, 12 h, 24 h, 48 h and 120 h after admission in plasma of cardiogenic shock patients. (*A*) cDPP3 concentrations measurements via LIA of 90-day survivors vs. non-survivors at baseline, 12 h, 24 h, 48 h and 120 h. In order to have identical patient numbers for each box, missing data were replaced by carrying the last observed value forward. (*B*) Correlation curve between DPP3 activity measurements via ECA and DPP3 concentration measurements via LIA ($r > 0.99$, $P < 0.001$).

Figure S2. Individual data on the shortening fraction of mice before and 15 min after cDPP3 injection. Comparison was made by Mann–Whitney test, $P < 0.05$ was considered significant.

Figure S3. ISO, isoproterenol; Characterization of HF mice model and effect of PCZ on cardiac function until 14 days of ISO protocol. (*A*) Cardiac output in sham and isoproterenol-injected (ISO) mice 12 h after the last injection of isoproterenol. Comparison was made by Mann–Whitney test. (*B*) Lung weight on tibia length rapport in sham and isoproterenol-injected (ISO) mice 12 h after the last injection of isoproterenol. Comparison was made by Mann–Whitney test. (*C*) E/A ratio in sham and isoproterenol injected (ISO) mice 12 h after the last injection of isoproterenol. Comparison was made by Mann–Whitney test. (*D*) mRNA DPP3 in heart of sham and isoproterenol-injected (ISO) mice 12 h after the last injection of isoproterenol. Comparison was made by Mann–Whitney test. (*E*) DPP3/GADPH immunoblot in heart of sham and isoproterenol-injected (ISO) mice 12 h after the last injection of isoproterenol. Comparison was made by Mann–Whitney test. (*F*) Evolution of shortening fraction after isoproterenol injection and PBS injection (vertical dotted line, $n = 5$), PCZ ($n = 5$) and non-active IgG ($n = 3$) at day 3. Mice were monitored during 14 days after the beginning of isoproterenol injection with haemodynamics point at day 8 and 14. Animals were sacrificed at day 14.

Figure S4. ISO, isoproterenol; Cardiac and renal effects of PCZ in ISO-HF mice. (*A*) Individual data on the shortening fraction of ISO-HF + PCZ mice before and 1 h after PCZ injection. Comparison was made by Wilcoxon paired test. (*B*) Evolution of shortening fraction in Sham + PBS and Sham + PCZ group, 1 and 6 h after injection of PBS or PCZ. Comparison was made by repeated measures of ANOVA, data shown as mean ± SD. (*C*) Isoproterenol protocol and images from echocardiography and kidney ultrasound at baseline, day 3 after the beginning of ISO injections (12 h after the last injection of isoproterenol) and 24 h after PCZ injection in mice. (HF: heart failure, ISO: isoproterenol, PCZ: Procizumab.)

Figure S5. ISO, isoproterenol; Characterization of DPP3 inhibiting antibody PCZ. (*A*) Binding sensogram of the captured PCZ interacting with GST-hDPP3 (3.7, 11.1, 33 and 100 nM dilutions) and 1:1 kinetic model fit overlays in the Octet RED96. Only

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association and dissociation steps are shown, divided by a vertical line. The processed data curves represent non-linear regression fits from 1:1 binding model and global analysis. Global fitting results: ka = 8.4 × 104 M-1 s-1; kd = 8.1 × 10−4 s-1 yielding a KD = 9.6 nM. Goodness of fit R2 = 0.9994. (*B*) Western blot specificity analysis of PCZ in human (hBCL) and murine blood cell lysates, heart, and kidney homogenates (mBCL). PCZ identifies a single band between 75 and 80 kDa in western blot. As control, human DPP3 natively purified from blood cell lysate was used (hDPP3). (*C*) Inhibition curve of PCZ with natively purified hDPP3. DPP3 inhibition by PCZ was investigated using the SAA assay in the presence of a fluorogenic substrate. PCZ binds hDPP3 and inhibits hDPP3 cleavage of Arg2-βNA, leading to reduced fluorescence. Non-linear regression fitting resulted in IC50 = 7.05 μg/mL and Imax = 73.3%.

Table S1. Angiotensin levels in human and mice plasma.

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Conflict of interest: A.M. reports personal fees from Novartis, Orion, Roche, Servier, Sanofi, Otsuka, Philips, grants and personal fees from Adrenomed, Abbott, and grants from 4TEEN4. In addition A.M. owns fewer than 3000 euros shares of S-Form Pharma company. B.D. and A.Bl. were invited to a meeting in Henningsdorf by 4TEEN4 Pharmaceuticals GmbH. K.S. and A.D. are employed at 4TEEN4 Pharmaceuticals. A.B. is CEO and shareholder at 4TEEN4 Pharmaceuticals. 4TEEN4 owns intellectual properties related to the therapeutic use of DPP3 inhibitors and diagnostic use of DPP3. The remaining authors declared no potential conflicts of interest with respect to the research authorship and/or publication of this article.

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