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Myeloperoxidase Inhibition Reverses Biomarker Profiles Associated With Clinical Outcomes in HFpEF

Erik Michaëlsson, PhD,[a] Lars H. Lund, MD, PhD,[b] Camilla Hage, PhD,[b,c] Sanjiv J. Shah, MD, PhD,[d] Adriaan A. Voors, MD, PhD,[e] Antti Saraste, MD, PhD,[f] Björn Redfors, MD, PhD,[g,h] Erik L. Grove, MD, PhD,[i,j] Anders Barasa, MD, PhD,[k] A. Mark Richards, MD, PhD,[l,m] Sara Svedlund, MD, PhD,[g,h] Maria Lagerström-Fermér, PhD,[a] Anders Gabrielsen, MD, PhD,[a] Pavlo Garkaviy, MD, PhD,[a] Li-Ming Gan, MD, PhD,[g,h] Carolyn S.P. Lam, MBBS, PhD[e,n]

ABSTRACT

BACKGROUND Systemic microvascular dysfunction and inflammation are postulated to play a pathophysiologic role in heart failure with preserved ejection fraction (HFpEF).

OBJECTIVES This study aimed to identify biomarker profiles associated with clinical outcomes in HFpEF and investigate how inhibition of the neutrophil-derived reactive oxygen species–producing enzyme, myeloperoxidase, affects these biomarkers.

METHODS Using supervised principal component analyses, the investigators assessed the associations between baseline plasma proteomic Olink biomarkers and clinical outcomes in 3 independent observational HFpEF cohorts (n = 86, n = 216, and n = 242). These profiles were then compared with the biomarker profiles discriminating patients treated with active drug vs placebo in SATELLITE (Safety and Tolerability Study of AZD4831 in Patients With Heart Failure), a double-blind randomized 3-month trial evaluating safety and tolerability of the myeloperoxidase inhibitor AZD4831 in HFpEF (n = 41). Pathophysiological pathways were inferred from the biomarker profiles by interrogation of the Ingenuity Knowledge Database.

RESULTS TNF-R1, TRAIL-R2, GDF15, U-PAR, and ADM were the top individual biomarkers associated with heart failure hospitalization or death, and FABP4, HGF, RARRES2, CSTB, and FGF23 were associated with lower functional capacity and poorer quality of life. AZD4831 downregulated many markers (most significantly CDCP1, PRELP, CX3CL1, LIFR, VSIG2). There was remarkable consistency among pathways associated with clinical outcomes in the observational HFpEF cohorts, the top canonical pathways being associated with tumor microenvironments, wound healing signaling, and cardiac hypertrophy signaling. These pathways were predicted to be downregulated in AZD4831 relative to placebo-treated patients.

CONCLUSIONS Biomarker pathways that were most strongly associated with clinical outcomes were also the ones reduced by AZD4831. These results support the further investigation of myeloperoxidase inhibition in HFpEF.
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From the [a]Early Clinical Development, Research and Early Development, Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden; [b]Department of Medicine, Cardiology Unit, Karolinska Institutet, Stockholm, Sweden; [c]Department of Cardiology, Karolinska University Hospital, Stockholm, Sweden; [d]Division of Cardiology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA; [e]University of Groningen, Groningen, the Netherlands; [f]Heart Center, Turku University Hospital, University of Turku, Turku, Finland; [g]Department of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden; [h]Department of Molecular and Clinical Medicine, Institute of

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ABBREVIATIONS AND ACRONYMS

6MWD = 6-minute walk distance

EF = ejection fraction

HbA_{1c} = glycosylated hemoglobin

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

KCCQ-OSS = Kansas City Cardiomyopathy Questionnaire Overall Symptoms Score

MPO = myeloperoxidase

NT-proBNP = N-terminal pro-B-type natriuretic peptide

OPLS = orthogonal projection to latent structures

OPLS-DA = orthogonal projection to latent structures with discriminant analysis

Systemic inflammation and microvascular dysfunction are postulated to play a key pathophysiologic role in heart failure (HF).[1] They may be a predominant driver of disease, particularly in patients with mildly reduced/preserved ejection fraction (EF), where comorbidities, as well as the HF syndrome per se, drive systemic inflammation, coronary microvascular dysfunction, left ventricular diastolic dysfunction, and left ventricular fibrosis, together resulting in the clinical syndrome of HF. Whereas several studies support the presence of microvascular dysfunction and systemic inflammation in patients with heart failure with preserved ejection fraction (HFpEF),[2-5] no clinical studies have convincingly demonstrated that inflammation in HFpEF may be modifiable with anti-inflammatory therapies.

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We recently tested the hypothesis that targeting systemic microvascular inflammation with the novel selective myeloperoxidase (MPO) inhibitor AZD4831 in HFpEF would be safe and would result in effective target engagement, assessed by MPO activity from whole blood stimulated with zymosan ex vivo.[6] MPO is released by neutrophils and activated by hydrogen peroxide, generating the strong oxidant hypochlorous acid that in turn can act on a broad number of protein, lipid, and nucleic acid targets, causing cellular and tissue dysfunction and further inflammation.[7] In addition, activated MPO consumes nitric oxide, which is one of the suggested mechanisms behind MPO-driven endothelial dysfunction.[8] By irreversibly inhibiting the hydrogen peroxide-activated redox forms of the enzyme, AZD4831 reduces free radical production, potentially breaks the cycle of microvascular dysfunction, and potentially inhibits and even reverses cardiac dysfunction and could thus be beneficial in patients with HFpEF.

Accordingly, we aimed to identify biomarker profiles associated with clinical outcomes in HFpEF and investigate how MPO inhibition may affect these biomarkers.

METHODS

Detailed descriptions of the methods are provided in the Supplemental Methods.

In 3 independent prospective observational HFpEF cohorts, KaRen (Karolinska-Rennes [KaRen] Prospective Study of Exercise Stress Echocardiography in Heart Failure With Preserved Ejection Fraction, NCT00774709; Swedish biomarker substudy),[9] SHOP (Singapore Heart Failure Outcomes and Phenotypes; ACTRN12610000374066) study,[10] and PROMIS-HFpEF (Prevalence of Microvascular Dysfunction in Heart Failure With Preserved Ejection Fraction) study,[3] we first examined the association of proteomic Olink biomarkers with clinical outcomes (HF hospitalization or death) during follow-up, exercise capacity by 6-minute walk distance (6MWD), and quality of life by Kansas City Cardiomyopathy Questionnaire Overall Symptoms Score (KCCQ-OSS) at baseline, in patients with HFpEF. Next, based on the identified proteomic patterns, we discerned pathophysiologically important morbidity- and mortality-associated pathways using the Ingenuity Knowledge Database.[11] Finally, we explored whether MPO inhibition with AZD4831 in the SATELLITE trial modulated these key pathophysiological mechanisms in patients with HFpEF.

COHORTS. The KaRen study was a prospective observational multicenter study[9] in which the prespecified KaRen biochemistry substudy enrolled 86 patients with an EF ≥45% at Karolinska University Hospital who were followed up for a median of 579 days.

The SHOP study[10,12] was a prospective observational multicenter study enrolling consecutive in- and outpatients with clinically confirmed HF from 6 centers in Singapore. A subset of patients had Olink measurements, including 219 patients with HFpEF, of whom 3 were excluded from the present analysis: 2 patients because >50% of their data were missing, and 1 patient was an extreme outlier in the

Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; [i]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark; [j]Faculty of Health, Aarhus University, Aarhus, Denmark; [k]Department of Medicine, Glostrup Hospital, Copenhagen, Denmark; [l]Department of Medicine, University of Otago, Christchurch, New Zealand; [m]National University Heart Centre Singapore (NUHCS), National University of Singapore, Singapore; and the [n]National Heart Centre Singapore, Duke-National University of Singapore, Singapore.

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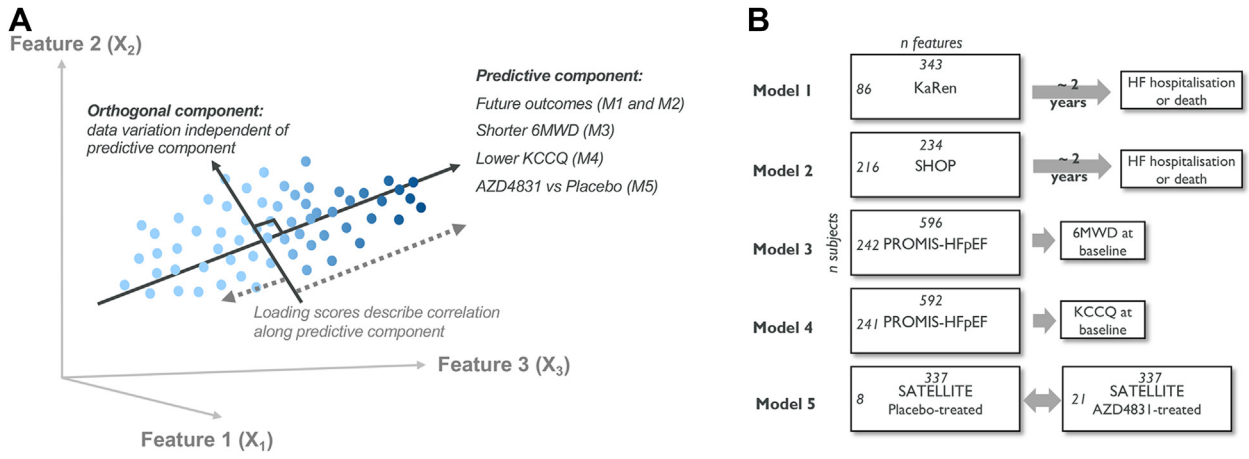
FIGURE 1 **Principle and Overview of the OPLS Models Generated**



(A) Illustration of the principle of the orthogonal projection to latent structures (OPLS) analysis, in which the predictive principal component distills out how all the variables relate to what endpoint is modeled (predicted). The "projection" of each variable (feature) along the predictive component is represented by a loading score (length and directionality of **dashed arrow**) that quantifies how well the variable associates with what is modeled. The orthogonal components, on the other hand, capture significant data variations independent of the predictive component, "noise." **(B)** The 5 models that were generated from the 4 different cohorts. The **boxes** with cohort names illustrate the modeled data matrices, in which the number of patients is indicated in the vertical direction and the number of variables (features) in the horizonal direction. 6MWD = 6-minute walk distance; HF = heart failure; KaRen = Karolinska-Rennes Prospective Study of Exercise Stress Echocardiography in Heart Failure With Preserved Ejection Fraction; KCCQ = Kansas City Cardiomyopathy Questionnaire; PROMIS-HFpEF = Prevalence of Microvascular Dysfunction in Heart Failure With Preserved Ejection Fraction; SATELLITE = Safety and Tolerability Study of AZD4831 in Patients With Heart Failure; SHOP = Singapore Heart Failure Outcomes and Phenotypes study.

orthogonal projection to latent structures (OPLS) model.

The PROMIS-HFpEF study[3] was a prospective study enrolling patients with a confirmed diagnosis of chronic HF with EF ≥40%, at 5 centers in Sweden, Finland, the United States, and Singapore. Study procedures included a history and physical examination, fasting blood and urine testing, 6MWD test, KCCQ score, and comprehensive echocardiography.

SATELLITE was a randomized, double-blind, placebo-controlled, multicenter, phase 2a study in patients with HFpEF (EF ≥40%) that evaluated safety and target engagement (MPO activity) of 90-day treatment with oral AZD4831, designed as 2 parts (A and B, separated by an interim analysis based on safety and target engagement). Planned interim analysis, after the completion of part A, was performed with 37 randomized patients, of whom 32 completed 90 days of study treatment. Shortly after initiation of part B, the study was temporarily halted and study treatment was stopped, because of the COVID-19 pandemic. Of the 14 and 27 patients randomized to placebo and AZD4831 treatment in the SATELLITE study, 10 discontinued study treatment (6 on placebo and 4 on AZD4831 treatment). Of the remaining 8 patients treated with placebo and 23 treated with AZD4831, 90-day baseline-adjusted data were available for 8 patients who were placebo-treated and 21 who were AZD4831-treated, which were all included the current analyses.

In all cohorts and in the SATELLITE trial, the studies were approved by relevant ethics authorities, and all patients provided written informed consent. KaRen was approved by Regionala etikprövningsnämnden i Stockholm. SHOP was approved by the National Healthcare Group Domain Specific Review Board, Singapore. PROMIS and SATELLITE were approved by Regionala etikprövningsnämnden i Stockholm (Sweden), Singhealth Centralised Institutional Review Board C (Singapore), De Videnskabsetiske Komitéer (Denmark), National Committee on Medical Research Ethics (Finland), Foundation Assessment Ethics Biomedical Research (the Netherlands), and Northwestern University Institutional Review Board (United States).

VARIABLES. The variables comprised demographics, morphometrics, echocardiography, comorbidities (and variables thereof, eg, glycosylated hemoglobin

TABLE 1 Baseline Characteristics of KaRen, SHOP-HFpEF, PROMIS-HFpEF, and SATELLITE Trials

	KaRen (n = 86)			SHOP-HFpEF (n = 216)			PROMIS-HFpEF (n = 242)			SATELLITE (n = 41)		
	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3
Age, y	73	67	79	69	60	77	75	70	81	74	72	79
Female	44 (51)			114 (53)			137 (57)			19 (46)		
White	86 (100)			0			210 (87)			40 (98)		
Black	0			0			10 (4)			1 (2)		
Asian	0			217 (100)			22 (9)			0		
Chinese	0			139 (64)			0			0		
Indian	0			20 (9)			0			0		
Malay	0			55 (25)			0			0		
Previous diagnosis of HF	30 (35)			187 (87)			239 (99)			41 (100)		
HF diagnosis >1 y	12 (14)			35 (20)			117 (48)			36 (88)		
Duration of HF, y	0	0	0	ND			1	0	4	NA		
NYHA functional class												
I	19 (22)			51 (24)[a]			3 (1)			0		
II	47 (55)			132 (62)[a]			178 (73)			NA		
III	20 (23)			27 (13)[a]			60 (25)			NA		
IV				3 (1)[a]			1 (0.5)			NA		
Medical history												
Ischemic heart disease	29 (34)			73 (34)			98 (41)			18 (40)		
Hypertension	66 (77)			186 (86)			199 (82)			35 (85)		
Atrial fibrillation	52 (60)			67 (31)			132 (55)			25 (61)		
Diabetes	28 (33)			126 (58)			69 (29)			10 (24)		
Hyperlipidemia				NA			113 (47)			20 (49)		
Smoker (current or previous)	45 (58)			62 (29)			162 (67)			15 (37)		
Physical characteristics												
Systolic BP, mm Hg	140	130	150	131	119	144	140	125	153	144	130	157
Diastolic BP, mm Hg	80	70	85	68	60	78	77	69	85	82	75	90
Heart rate, beats/min	70	60	80	70	62	80	68	60	78	66	57	76
BMI, kg/m^2	28.5	25.0	32.9	26.6	23.7	30.3	28.2	24.4	32.5	27.6	252	31.4
Obese BMI ≥30 kg/m^2/>25 kg/m^2 in Asia	35 (41)			141 (67)			85 (35)			13 (32)		

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[HbA_{1c}], lipoproteins), medication, symptoms, physical findings, vital signs, clinical chemistry, and Olink proteomic data generated from EDTA-plasma samples drawn at enrollment in the observational cohorts and at randomization and after 90 days of treatment in the SATELLITE study. Olink data are based on a multiplexed proximity extension assay, yielding normalized protein expression semi-quantitative data on a $\log_2$ scale. The Olink Target 96 Cardiovascular (CVD[I]) panel (92 biomarkers) was used for the KaRen and SHOP trials, and a combination of the 3 92-plex panels Olink Target 96 Cardiovascular II, III, and Inflammation (266 unique biomarkers) were used for the PROMIS-HFpEF and SATELLITE trials. All but 8 of the biomarkers represented in the Olink Target 96 Cardiovascular panel are covered by these 3 panels: CA125 (*MUC16*), ECP (*RNASE3*), EGF (*EGF*), ESM1 (*ESM1*), GAL (*GAL*), hK11 (*KLK11*), mAmP (*XPNPEP2*), and PRL (*PRL*). Supplemental Table 1 contains the complete list of biomarkers, genes, full protein names, and the panel overlap.

PRINCIPAL COMPONENT ANALYSES AND OTHER STATISTICAL ANALYSES. We used supervised principal component analysis (OPLS)[13] (SIMCA 15, Umetrics suite; Sartorious) as a tool to investigate the relationship between multiple features of the multifactorial syndrome of HFpEF. This tool condenses a large, multidimensional data matrix into 1 or a few dimensions (**Figure 1A**) and is commonly used in machine learning and "big data" sciences to extract the most meaningful variables describing complex data. The number of patients and the diagnostics of the respective models is summarized in **Figure 1B** and Supplemental Table 2.

For the observational cohorts containing outcome data (KaRen and SHOP), OPLS models were generated that described the relationship between baseline data and clinical events (HF hospitalization or death) in KaRen (n = 86; median follow-up time:

TABLE 1 Continued

	KaRen (n = 86)			SHOP-HFpEF (n = 216)			PROMIS-HFpEF (n = 242)			SATELLITE (n = 41)		
	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3	Median or n (%)	Quartile 1	Quartile 3
Echocardiographic characteristics												
LVEF, %	64	58	68	60	55	63	59	55	64	52	47	57
LVEF ≥50%	73 (97)			212 (98)			213 (88)			25 (64)[a]		
Septal wall thickness, cm	1.1	1.0	1.3	1.1	1.0	1.3	1.3	1.1	1.5	1.1	0.87	1.2
LV mass index, g/m^2	114	95	143	113	91	141	103	83	124	88	71	101
LVH	25 (61)			121 (60)			128 (53)			NA		
Cardiac output, L/min	5.0	4.3	6.0	3.1	2.4	3.9	4.5	3.7	5.5	4.1	3.4	4.7
LV global longitudinal strain, %	15.0	12.0	17.5	NA			16.5	13.3	18.4	13.5	13.1	17.7
LAVI, mL/m^2	44	38	52	35	27	51	38	30	45	54	42	65
LAVI >34 mL/m^2	66 (89)			101 (52)			139 (57)			35 (92)[a]		
LA reservoir strain, %	11.0	4.1	20.0	NA			12.8	9.2	22.4	8.5	5.2	15.7
TAPSE, mm	16.5	13.0	20.0	NA			17.7	15.7	20.7	19.6	15.4	23.0
LV E/e′ ratio	11	8	14	15	12	20	12	9	16	11	8.5	15
PCWP, mm Hg	NA			NA			18.0	16.4	19.7	NA		
Laboratory												
NT-proBNP, pg/mL	1,000	469	2,330	970	371	2,315	974	360	1,780	1,111	543	1,655
eGFR, mL/min/1.73 m^2	70	54	85	56	40	79	60	46	72	70	59	82
UACR, mg/g				NA			2.8	1.3	8.6	NA		
Hb, g/dL	131	122	142	117	101	132	129	118	139	134	126	152
Potassium, mmol/L	3.9	3.7	4.2	4.1	3.8	4.5	4.2	3.9	4.4	4.3	4.0	4.5
Sodium, mmol/L	141	140	143	139	136	141	140	138	142	140	139	142
Troponin T, ng/mL	NA			22	14	41	13	10	21	13	8.8	21
HbA_{1c}, mmol/mol	NA			NA			41	38	49.5	41	38	46
Glucose, mmol/L	5.6	5.1	7.5	NA			5.8	5.3	6.9	5.7	5.3	6.2
HOMA IR	3.4	2.0	5.6	NA			2.4	1.5	4.6	1.3	0.9	2.6
Cholesterol, mmol/L	NA			NA			4.2	3.47	4.9	4.1	3.6	4.7
LDL, mmol/L	NA			NA			2.2	1.7	2.9	2.0	1.7	2.8
Triglycerides, mmol/L	NA			NA			1.1	0.82	1.6	1.0	0.78	1.5
Treatment												
ACE/ARB/ARN inhibitors	67 (78)			170 (78)			129 (53)			40 (98)		
Beta-blocker	69 (80)			177 (82)			182 (75)			36 (88)		
MRA	18 (21)			27 (13)			66 (27)			7 (17)		
Loop diuretic agents	61 (71)			175 (81)			115 (48)			21 (51)		
Lipid-lowering	38 (44)			NA			137 (57)			30 (73)		
Glucose-lowering	23 (27)			NA			64 (26)			13 (32)		
Anticoagulant	47 (55)			NA			45 (18)			19 (46)		
Pacemaker	13 (15)			6 (2.8)			40 (17)			NA		
Outcomes												
KCCQ-OSS				NA			67	47	82	75	61	87
6MWD, m				NA			332	222	411	400	325	450
Composite HF hospitalization and all-cause mortality	36 (42)			76 (36)			21 (9)			NA		
HF hospitalization	30 (35)			59 (27)			16 (7)			NA		
All-cause mortality	6 (7)			24 (11)			7 (3)			NA		
Median follow-up time, d	522	238	1,089	721	391	730	389	365	420	NA		

[a]NYHA functional class available for 212 patients in the SHOP cohort. EF data available for 39 patients in the SATELLITE cohort. LAVI data available for 38 patients in the SATELLITE cohort. eGFR calculated by MDRD in the KaRen and SHOP trials and by CKD-EPI in the PROMIS-HFpEF and SATELLITE trials.

6MWD = 6-minute walk distance; ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; ARN = angiotensin receptor–neprilysin; BMI = body mass index; BP = blood pressure; CKD-EPI = CKD Epidemiology Collaboration; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HbA_{1c} = glycosylated hemoglobin; HF = heart failure; HOMA IR = homeostatic model assessment for insulin resistance; KaRen = Karolinska-Rennes Prospective Study of Exercise Stress Echocardiography in Heart Failure With Preserved Ejection Fraction; KCCQ-OSS = Kansas City Cardiomyopathy Questionnaire Overall Symptoms Score; LA = left atrial; LAVI = left atrial volume index; LDL = low-density lipoprotein; LV = left ventricular; LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; MDRD = Modification of Diet in Renal Disease; MRA = magnetic resonance angiography; NA = not available; ND = not determined; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCWP = pulmonary capillary wedge pressure; PROMIS-HFpEF = Prevalence of Microvascular Dysfunction in Heart Failure With Preserved Ejection Fraction; SATELLITE = Safety and Tolerability Study of AZD4831 in Patients With Heart Failure; SHOP-HFpEF = Singapore Heart Failure Outcomes and Phenotypes in Heart Failure With Preserved Ejection Fraction; TAPSE = tricuspid annular plane systolic excursion; UACR = urinary albumin-to-creatinine ratio.

579 days, model 1) and SHOP-HFpEF (n = 216; 2-year follow-up, model 2). To identify correlation to functional capacity and quality of life, the relationship between baseline data and 6MWD (n = 242, model 3) and KCCQ-OSS,[14] respectively, were modeled in the PROMIS-HFpEF cohort. KCCQ data were available for 252 patients, of whom 241 met the criteria for inclusion in the OPLS model (model 4). AZD4831 treatment effects in the SATELLITE cohort were modeled by a discriminant analysis (OPLS-DA) comparing baseline-adjusted 3-month data (ie, absolute change from baseline) between patients treated with placebo (n = 8) and those treated with AZD4831 (n = 21) (model 5), representing all patients with paired baseline and 3-month Olink data.

In summary, 5 different OPLS models were generated (**Figure 1B**) for HF hospitalization or all-cause mortality in the KaRen trial (model 1) and the SHOP trial (model 2), poor exercise capacity (shorter 6MWD, model 3) and poor quality of life (lower KCCQ-OSS score, model 4) in the PROMIS-HFpEF trial, and baseline-adjusted 90-day placebo vs AZD4831-treatment associated data in the SATELLITE trial (model 5).

Olink biomarker results (baseline-subtracted 3-month normalized protein expression values) were compared between placebo- and AZD4831-treated individuals (Supplemental Table 3) using unpaired Student's *t*-test with Welch correction, not corrected for multiple comparisons (GraphPad Prism 9, GraphPad Software). To compare the ranked outcome association of the biomarkers to similar data previously reported from other cohorts, we used Spearman's rank test (2-sided *P* value) (GraphPad Prism 9) to compare outcome-associated biomarker OPLS correlation coefficients (ie, mean of normalized loading values in the KaRen and SHOP trials) with the unadjusted HRs for HF hospitalization or death in TOPCAT (Aldosterone Antagonist Therapy for Adults With Heart Failure and Preserved Systolic Function)[5] and risk ratios for incident HF.[15]

IDENTIFICATION OF PATHOPHYSIOLOGICAL MECHANISMS FROM BIOMARKER PATTERNS. To investigate pathways potentially associated with morbidity and mortality and targeted by AZD4831 treatment, respectively, biomarker patterns were compared with data curated from the public domain (Ingenuity Knowledge Database).[11] This was done by using the normalized correlation coefficients of significantly contributing biomarkers, obtained in the OPLS (and OPLS-DA) models, as input (as log-fold expression) for the Ingenuity Pathway Analysis core analysis. Based on the overlap of biomarkers annotated to the mechanism and the up- or down-regulation of the biomarkers, the analysis predicts the involvement and directionality of canonical pathways and identity of likely upstream regulators. To compare the identified regulators and pathways in OPLS models 1-5, a comparison analysis of the 5 models was performed in Ingenuity Pathway Analysis and the result was ranked by *z*-scores.

Further detailed Methods are presented in the Supplemental Methods.

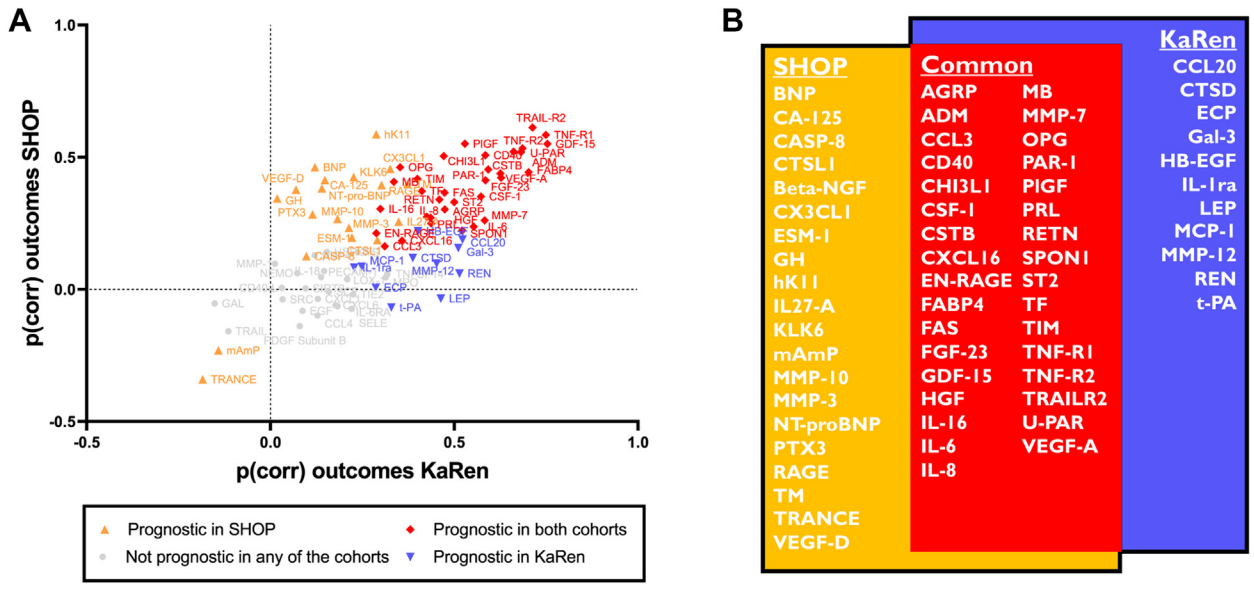
RESULTS

Baseline characteristics of the 3 HFpEF cohorts and the HFpEF randomized, controlled trials are summarized in **Table 1**. Overall, the included patients were typically elderly women with a high prevalence of hypertension and atrial fibrillation, symptomatic HF (primarily NYHA functional class II), as well as evidence of structural and functional heart disease (left ventricular hypertrophy, left atrial enlargement, increased mitral E/e′ ratio), and increased natriuretic peptides.

BIOMARKERS ASSOCIATED WITH FUTURE HF HOSPITALIZATION OR DEATH IN THE KaRen AND SHOP TRIALS. **Figure 2A** compares how each of the Olink biomarkers associate with outcomes in the OPLS models predicting the composite of HF hospitalization or all-cause mortality in the KaRen (model 1) and SHOP (model 2) trials. Of the 92 Olink biomarkers assessed, 33 were positively and significantly associated with the composite outcome in both cohorts (red in **Figures 2A and 2B**). The 5 strongest markers were TNF-R1, TRAIL-R2, GDF15, U-PAR, and ADM (top right of **Figure 2A**). Eleven and 20 biomarkers were uniquely associated with the composite outcome in the KaRen and SHOP trials, respectively (blue and orange in **Figure 2B**, and see also Supplemental Table 1).

BIOMARKERS ASSOCIATED WITH POOR FUNCTIONAL CAPACITY AND POOR QUALITY OF LIFE IN THE PROMIS-HFpEF TRIAL. **Figure 3** compares how each of the Olink biomarkers associated with shorter 6MWD (model 3) and lower quality of life (model 4), respectively, in the PROMIS-HFpEF trial. Of the 266 unique Olink biomarkers, 16 were significantly associated with both 6MWD and KCCQ-OSS (red). Lower levels of GH and PON3 and higher levels of CSTB, FABP4, FGF21, FGF23, HGF, IL18R1, IL6, MMP9, OSM, PLC, RARRES2, tissue-type plasminogen activator (tPA), transferrin receptor protein 1 (TR), and VEGFA were all associated with a lower KCCQ-OSS and

FIGURE 2 **Association Between Baseline CVD[I] Olink Biomarkers and Future Outcomes Are Similar in the KaRen and SHOP Trials**



The association between baseline Olink Target 96 Cardiovascular (CVD[I]) biomarkers and future outcomes is similar in the KaRen and SHOP trials. **(A)** The relationship between baseline proteomic biomarkers and subsequent outcomes in the SHOP trial (x-axis) is compared with those in the KaRen trial (y-axis). Data are presented as normalized loading scores (p(corr)) in the OPLS models. Olink biomarkers with a significant contribution in both models are shown as **red diamonds**, in SHOP only as **orange triangles**, in KaRen only as **blue triangles**, and in none of the studies as **gray circles**. **(B)** Venn diagram shows the distribution of Olink biomarkers associated with outcomes in either or both of the cohorts. Abbreviations as in Figure 1.

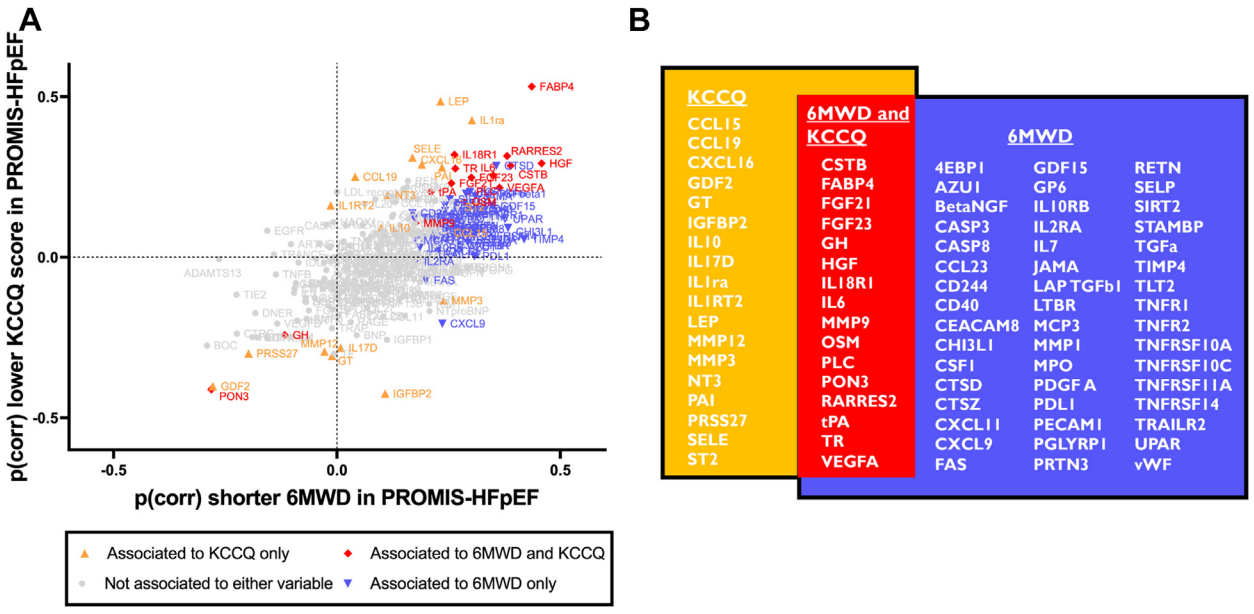
shorter 6MWD. Of these biomarkers, CSTB, FABP4, FGF23, GH, HGF, IL6, tPA, and VEGFA are represented in the Olink CVD[I] panel assessed in the KaRen and SHOP studies, all of which, except GH and tPA, were significantly associated with HF hospitalization or all-cause mortality in both cohorts (Figure 2). Additional biomarkers were associated only with 6MWD (blue) or only with KCCQ (orange).

Of the other patient features (data not shown), the OPLS analyses identified that a lower KCCQ-OSS and shorter 6MWD were associated with higher uric acid, HbA_{1c}, and neutrophil counts, dyspnea (post-6MWD and paroxysmal nocturnal dyspnea), New York Heart Association functional class, lung rales, mitral E wave velocity, and lower baseline coronary blood flow. Of note is that low estimated glomerular filtration rate and high E/e′ were among the top descriptors of shorter 6MWD, but had no descriptive value for KCCQ-OSS.

EFFECTS OF A RANDOMIZED PLACEBO-CONTROLLED MPO INTERVENTION ON OLINK BIOMARKERS. The effects of a 3-month treatment with the MPO inhibitor AZD4831 vs placebo on the 266 Olink biomarkers are illustrated in the volcano plot in Figure 4A, showing the effect size and significance for the individual markers. Forty-five of the individual biomarkers were significantly down-regulated (unadjusted $P < 0.05$) in patients treated with AZD4831 vs those treated with placebo, the 10 most significant ones being CDCP1, PRELP, CX3CL1, LIFR, VSIG2, PDL1, MMP10, PDL2, IL10RB, and PRSS27 (Figure 4A, Supplemental Table 3). None of the individual biomarkers were significantly up-regulated.

Next, we analyzed the biomarker pattern (rather than the individual biomarkers) associated with AZD4831 treatment by performing OPLS analysis comparing baseline-adjusted 90-day data (n = 337 variables, including Olink) between patients treated with placebo and those treated with AZD4831. Figure 4B shows the distribution of the 29 individual patients along the OPLS principal component describing the group difference, illustrating that the OPLS model could classify 11 and 4 of the patients as AZD4831- or placebo-treated, respectively. The rest of the patients either had a "nonresponder" or intermediate phenotype that was not captured by the OPLS separation with 95% certainty. In total, 176 of the 266 Olink biomarkers were contributing to the

FIGURE 3 Association Between Olink Biomarkers and Poor Exercise Capacity and Poor Quality of Life in PROMIS-HFpEF



(A) The relationship between proteomic biomarkers and shorter 6MWD (x-axis) is compared to the lower KCCQ score (y-axis). Data are presented as normalized loading scores (p(corr)) in the OPLS models. Olink biomarkers with significant contribution in both models are shown as **red diamonds**; those associated with shorter 6MWD only as **blue triangles**; those associated with lower KCCQ score only as **orange triangles**; and those with no association to either endpoint as **gray circles**. **(B)** Venn diagram shows the distribution of Olink biomarkers associated with either or both shorter 6MWD and lower KCCQ score. Abbreviations as in Figure 1.

OPLS separation (Supplemental Table 1), and the top-10 (TLT2, PRSS27, VEGFA, thrombomodulin (TM), CCL3, CX3CL1, LIFR, TWEAK, CD84, and AGRP) are illustrated in **Figure 4C**.

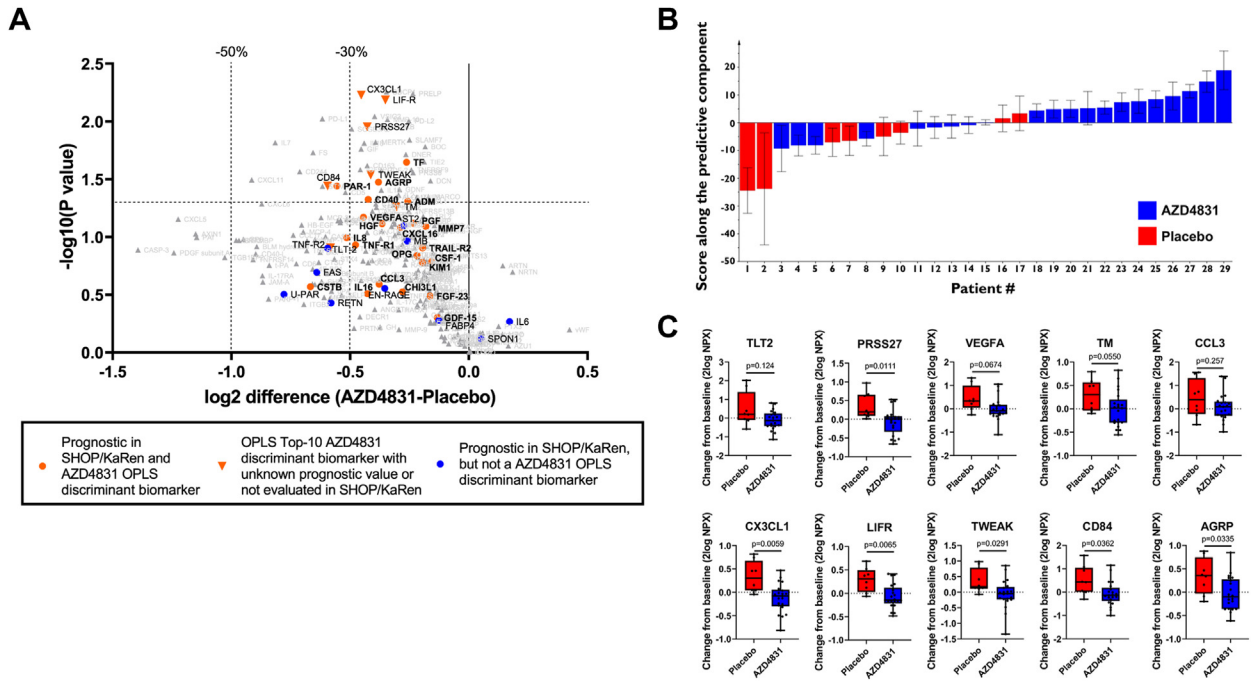
Of the 33 biomarkers that predicted HF hospitalization or all-cause mortality in the KaRen and SHOP trials, 22 contributed to the AZD4831 vs placebo separation (ADM, AGRP, CCL3, CD40, CHI3L1, CSF1, CSTB, CXCL16, FGF23, GDF15, HGF, IL16, IL8, KIM1, MMP7, OPG, PAR1, PGF, TF, TNFR1, TRAILR2, VEGFA) (**Figure 4A**). The full list of the OPLS-DA correlation coefficients along with the predictive component is shown in Supplemental Table 1, and the unadjusted *P* values and group differences are summarized in Supplemental Table 3.

COMPARISON OF PATHWAYS ASSOCIATED WITH CLINICAL OUTCOMES AND PATHWAYS INFLUENCED BY AZD4831. **Figure 5** shows a comparison of upstream regulators and canonical pathways inferred from the biomarker patterns associated with the different clinical outcomes in the observational HFpEF and AZD4831-treatment cohorts (see Supplemental Tables 4 and 5 for full lists). There was a remarkable consistency between pathways associated with eventual HF hospitalization or death in the KaRen (lane 1) and SHOP (lane 2) studies and with poor exercise capacity as well as with poor quality of life in the PROMIS-HFpEF study (lanes 3 and 4). The top canonical pathways positively associated (positive *z*-scores, red color, lanes 1-4) with these clinical outcomes were those associated with tumor microenvironments, wound healing signaling, and cardiac hypertrophy signaling. Notably, based on the proteomic effects, these pathways were all predicted to be down-regulated in patients who were treated with AZD4831 relative to those who were treated with placebo (negative *z*-scores, blue color, lane 5). Of major importance, this indicates that the pathways that were most strongly associated with HF hospitalization or all-cause death, shorter 6MWD, and lower KCCQ score were also the ones reduced by AZD4831.

DISCUSSION

We evaluated the associations among proteomic biomarkers and clinical outcome events (HF hospitalization and death), functional status (6MWD), and quality of life/health status (KCCQ scores) in 3 distinct observational HFpEF cohorts; identified potential key pathophysiologic pathways in HFpEF; and demonstrated a beneficial effect of the MPO inhibitor

FIGURE 4 Effect of AZD4831 on Individual Olink Biomarkers and OPLS-Based DA of Patients Receiving Placebo vs AZD4831 in the SATELLITE Study



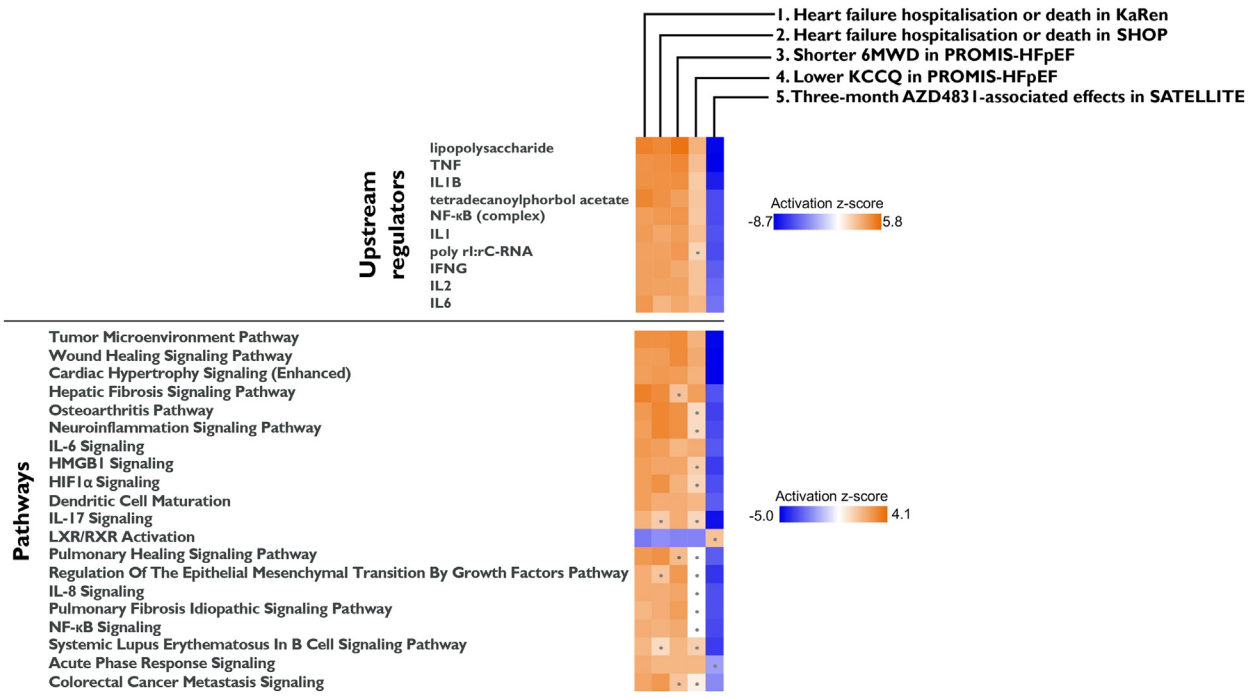
(A) Volcano plot of the 90-day Olink biomarker changes in the SATELLITE study; the x-axis shows the average AZD4831-placebo group difference in baseline-adjusted individual normalized protein expression (NPX) levels and the y-axis shows the −10log of the *P* value for the group difference. The **vertical dotted lines** represent 30% and 50% relative reductions in patients treated with AZD4831 vs those treated with placebo. **Circles** represent the 33 common outcome-predicting biomarkers identified from models 1 and 2, of which the **orange** represents whether the biomarker was among those discriminating patients who were placebo-treated from those who were AZD4831-treated in the OPLS model. **Orange triangles** represent the top-10 OPLS AZD4831- vs placebo-discriminating biomarkers with unknown prognostic value (CX3CL1 was prognostic in the SHOP study, but not the KaRen study; LIFR, PRSS27, TNF-related weak inducer of apoptosis [TWEAK], CD84, TM, TLT2 not represented in the CVD[I] panel). $P = 0.05$ (unadjusted) is highlighted by the **dashed horizonal line. (B)** The distribution (scores) of the 29 patients along the discriminant principal component (y-axis) in the OPLS separating the baseline-adjusted 90-day phenotype of the placebo-treated group **(red)** from the AZD4831-treated group **(blue)** in the SATELLITE study. The numerical values along the y-axis indicate how well the patients align to the annotated group (eg, patients 1 and 29 differ the most in the cohort, and patients 3, 4, 5 and 8, the 4 who were AZD4831-treated map as "nonresponders" among the patients who were placebo-treated). **(C)** Box and whiskers plots (median, Q1-Q3, minimum, maximum, as well as individual values) of the top-10 Olink biomarkers from the OPLS models separating the baseline-adjusted 90-day phenotype of the placebo-treated group from the AZD4831-treated group. DA = discriminant analysis; other abbreviations as in Figure 1.

AZD4831 on the identified biomarkers and pathways in a separate randomized trial (Central Illustration).

BIOMARKER CORRELATES OF OUTCOMES IN HFpEF: CONSISTENCY ACROSS COHORTS. Of the 33 protein biomarkers that predicted outcomes in the 2 geographically and ethnically distinct HFpEF cohorts (KaRen and SHOP), 6 were also associated with poor quality of life and poor functional capacity in a third independent observational cohort (PROMIS-HFpEF), and 22 were discriminant biomarkers for AZD4831 treatment (the SATELLITE trial). The prognostic value of the 33 Olink biomarkers are in agreement with a biomarker analysis (quantified by other technologies) in the TOPCAT HFpEF cohort,[5] of which 20 are represented in the Olink CVD[I] panel (Supplemental Figure 1A). Interestingly, the prognostic values of the Olink biomarkers reported here were also strikingly correlated ($P < 0.0001$) (Supplemental Figure 1B) to the risk ratio for incident HF (EF not reported) in a meta-analysis of 11,734 individuals.[15] Of note, whereas Olink N-terminal pro-B-type natriuretic peptide (NT-proBNP) was a significant predictor in the SHOP trial, it was not in the KaRen trial. One reason for this discrepancy of NT-proBNP may be the limited sensitivity of NT-proBNP quantification in the first-generation Olink CVD[I] panel, because NT-proBNP quantified by an enzyme-linked immunosorbent assay was indeed prognostic in the KaRen cohort.[16]

EFFECT OF MPO INHIBITION ON BIOMARKER PROFILES IN HFpEF. Several members of the TNFR superfamily were among the prognostic biomarkers,

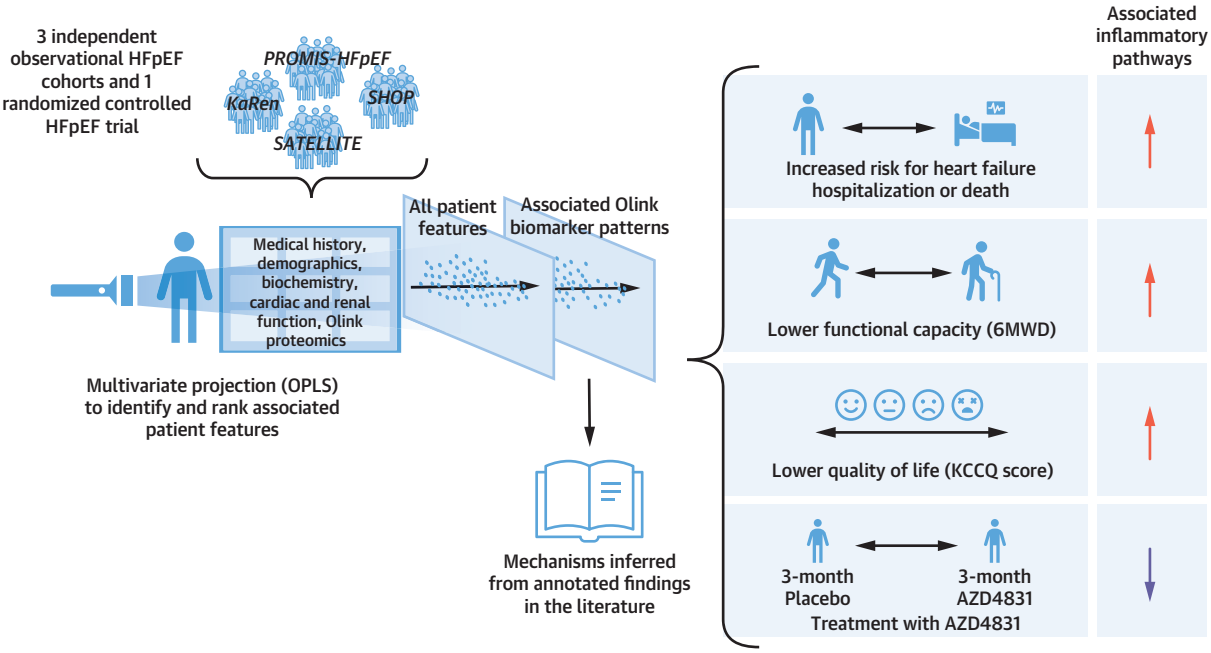
FIGURE 5 Comparison of Pathways and Upstream Regulators Associated With Future Outcomes, Impaired Functional Capacity, Poor Quality of Life, and MPO Intervention by AZD4831



The results represent a comparison of the outcome of the OPLS models (correlation coefficients for the significantly associated Olink biomarkers) with public data for these proteins, retrieved and annotated in the Ingenuity Knowledge Database.[11] The lanes represent biomarker patterns associated with risk for future hospitalization or death in the KaRen (lane 1) and SHOP (lane 2) trials; poor functionality assessed by 6MWD in the PROMIS-HFpEF trial (lane 3); poor quality of life assessed by KCCQ score in the PROMIS-HFpEF trial (lane 4); and placebo-corrected AZD4831 effects in the SATELLITE study (lane 5). The data are color graded for *z*-scores: the darker the color, the higher likelihood that the pathway/regulator is up-regulated **(orange)** or down-regulated **(blue).** A **dot** represents a nonsignificant association, meaning that the up- or down-regulation of the individual annotated biomarkers are not consistent with a directionality of the annotated function (numerical *z*-score <2). The full lists of upstream regulators, canonical pathways, and *z*-scores are shown in Supplemental Tables 4 and 5. HIF1α = hypoxia inducible factor 1, alpha subunit; HMGB I = high mobility group box 1; IFNG = interferon gamma; IL = interleukin; LXR = liver X receptor; MPO = myeloperoxidase; NF-kB = nuclear factor kappa B; rI:rC-RNA = polyinosinic:polycytidylic-RNA; RXR = retinoid X receptor; TNF = tumor necrosis factor; other abbreviations as in Figure 1.

and the top 2 (TNFR1 and TNFR2) likely originated from activated neutrophils.[17,18] That neutrophils are important in HF pathophysiology is also supported by the association of absolute numbers (or ratio to lymphocytes) with risk of incident HF,[19] poor outcomes independent of EF,[20] and a degranulating phenotype of neutrophils in HFpEF.[21] MPO is also released from neutrophils, and MPO concentrations are elevated in HF,[22,23] with a prognostic value in some cohorts and populations,[24,25] but not others.[5,23] How circulating concentrations of MPO relate to the turnover and the enzymatic activity of MPO in the tissues is currently not known. Yet, we were surprised to see how large a proportion of the 266 biomarkers appeared to be reduced by treatment with MPO inhibition relative to placebo. We considered other potential explanations for this broad impact on the proteome, in particular increased renal filtration, but we did not observe any consistent correlation between change of estimated glomerular filtration rate and change of biomarkers. Rather, we cautiously interpret the AZD4831 treatment effects on biomarkers in the SATELLITE trial as MPO activity being a central regulatory mechanism in the inflammatory pathophysiology, as illustrated by the Ingenuity Pathway Analysis knowledge base analysis presented in Figure 5 and also inferred from a prior network analysis.[26] Whether a smaller subset of these biomarkers may predict a treatment response to MPO inhibition and be clinically feasible for patient selection will require follow-up studies in larger cohorts. Until then, we consider elevated neutrophil counts as a feasible marker potentially predicting

CENTRAL ILLUSTRATION **Assessment of Proteomic Patterns Associated With Clinical Outcomes in Observational HFpEF Cohorts and Treatment With the MPO Inhibitor AZD4831 in the SATELLITE Trial**



Michaëlsson E, et al. J Am Coll Cardiol HF. 2023;11(7):775–787.

Patient features including Olink proteomic biomarkers associated with clinical outcomes (HF hospitalization and all-cause mortality, functional capacity, quality of life) were identified by a multivariate projection technique (orthogonal projection to latent structures [OPLS]) in 3 independent deeply phenotyped observational heart failure with preserved ejection fraction (HFpEF) cohorts (KaRen [Karolinska-Rennes Prospective Study of Exercise Stress Echocardiography in Heart Failure With Preserved Ejection Fraction], SHOP [The Singapore Heart Failure Outcomes and Phenotypes study]). The biomarkers were then compared with those associated with a 90-day placebo- and baseline-adjusted treatment with the MPO inhibitor AZD4831 in the randomized, multicenter, HFpEF phase 2a SATELLITE (Safety and Tolerability Study of AZD4831 in Patients With Heart Failure) trial that assessed safety, tolerability, and target engagement of AZD4831 in patients with HFpEF. The identity and directionality of likely upstream regulators and canonical pathways associated with the biomarker patterns were predicted using a knowledge database containing annotated findings based on full text articles. Clinical outcomes (functional capacity, quality of life, HF hospitalization, and all-cause mortality) were associated with proteomic patterns indicative of an inflammatory response similar to that observed in tumor microenvironments, wound healing, and cardiac hypertrophy. Myeloperoxidase inhibition partially reversed these pathways. 6MWD = 6-minute walk distance; HF = heart failure; KCCQ = Kansas City Cardiomyopathy Questionnaire; MPO = myeloperoxidase.

efficacy as well as identifying patients with a high medical need, as discussed herein.

STUDY LIMITATIONS AND STRENGTHS. First, this is a targeted and semiquantitative proteomic analysis using predefined biomarker panels, selected based on previously published cardiovascular and inflammation biomarker data, which therefore represent a bias in the identification of upstream regulators and pathways. Second, the cohorts are small and the interim analysis, after which the SATELLITE study was prematurely stopped, was powered for safety and target engagement but not for the post hoc exploratory Olink proteomics analyses presented herein. Our results are therefore limited by the small number of patients, which is balanced by the fact that the OPLS models were generated independently from each other and were consistent with biomarker studies in other HF cohorts. It should also be kept in mind that only one-half of the patients in the SATELLITE cohort could be classified as AZD4831- vs placebo-treated in the OPLS analysis comparing the 2 groups. Therefore, these data should be interpreted with caution. Yet, the consistency of biomarker profiling across diverse independent HFpEF cohorts and unique demonstration of the effect of a targeted anti-inflammatory approach on these biomarkers represent important hypothesis-generating data.

CONCLUSIONS

This study evaluated the association between proteomic biomarkers and clinical outcomes in 3 distinct observational HFpEF cohorts, compared with the biomarker effects observed after treatment with the MPO inhibitor AZD4831. Biomarker pathways most strongly associated with clinical outcomes were also the ones predicted to be downregulated after treatment with AZD4831. These results support further investigation of MPO inhibition in HFpEF.

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ADDRESS FOR CORRESPONDENCE: Dr Carolyn S.P. Lam, National Heart Centre Singapore, Duke-National University of Singapore, 5 Hospital Drive, Singapore 169609. E-mail: Carolyn.lam@duke-nus.edu.sg.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: We demonstrate an association between inflammatory pathways and morbidity/mortality, functional capacity and quality of life/health status in patients with HFpEF using comprehensive biomarker profiling in independent HFpEF cohorts. Inhibition of MPO activity inhibits these inflammatory pathways, thus supporting the hypothesis that MPO inhibition may translate into improved functional capacity, quality of life, and prognosis in patients with HFpEF.

TRANSLATIONAL OUTLOOK: The work adds to the evidence supporting inflammation as a potential target in HF with mildly reduced EF/HFpEF and builds evidence for the ongoing ENDEAVOR (Study to Evaluate the Efficacy and Safety of AZD4831 in Participants With Heart Failure With Left Ventricular Ejection Fraction >40%) trial evaluating the effects of AZD4831 on patient function and symptoms.

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KEY WORDS AZD4831, heart failure, HFpEF, inflammation, microvascular dysfunction, myeloperoxidase

APPENDIX For an expanded Methods section as well as supplemental tables and a figure, please see the online version of this paper.