Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Qualifying Criteria for Enrollment

Criterion to verify HFpEF diagnosis	**N (%)**[a]
Prior hospitalization for decompensated heart failure[b]	5 (5)
Elevated filling pressures at rest[c]	49 (47)
Elevated pressures during exercise, but normal at rest[d]	12 (11)
Elevated NT-proBNP (>400 pg/ml)	29 (28)
Elevated BNP (>200 pg/ml)	8 (8)
Echo-Doppler evidence of diastolic dysfunction with chronic loop diuretic[e]	48 (46)

[a]Participants may have qualified by more than one criterion.

[b]Hospitalization with unequivocal evidence of pulmonary congest as indicated by chest radiography and physical examination.

[c]Left ventricular filling pressures measured invasively at cardiac catheterization, with either pulmonary capillary wedge pressure≥15 mmHg and/or left ventricular end diastolic pressure ≥18 mmHg.

[d]Normal filling pressures measured invasively at catheterization at rest, but elevation during exercise defined as pulmonary capillary wedge pressure ≥25 mmHg.

[e]Defined as medial E/e' ratio ≥15 and/or left atrial enlargement in tandem with chronic treatment with a loop diuretic

eTable 2. Patient Characteristics in Comparison to Other Contemporary Trials in HFpEF

	NEAT-HFpEF N=110	**SOCRATES Preserved N=477**	**RELAX N=216**	**TOPCAT (Americas) N=1767**	**PARA-MOUNT N=152**	**INDIE-HFpEF N=105**
Age, years	69	73	69	72	71	68
Female sex, %	57	48	48	50	57	56
BMI, kg/m^2	35	30	33	33	30	35
Hypertension, %	90	-	85	90	95	81
Atrial fibrillation, %	35	40	51	42	40	45
Diabetes, %	39	49	43	45	41	35
NT-proBNP, pg/ml	227	1174	700	900	828	499
LVEF, %	64	57	60	58	58	61
E/e'	14	-	16	15	12	17[a]
LAVI, ml/m^2	37	43[b]	44	28	35	38[a]

[a]Values following treatment with placebo because baseline echocardiogram was not performed as part of the trial protocol.

[b]LA volume index calculated from the mean LA volume and body surface area reported.

eTable 3. Hemodynamic Effects of Nitrite vs Placebo

End Point	Nitrite (n=105)	Placebo (n=105)	Adjusted Treatment Difference[a]	P value
Systolic BP (mmHg)				
Pre-drug, rest[b]	130 (17)	125 (16)	5 (2, 9)	0.001
Post-drug, rest[c]	121 (16)	124 (16)	-3 (-7, 1)	0.10
Change with drug[d]	-9 (14)	-2 (13)	-7 (-11, -4)	<0.0001
Diastolic BP (mmHg)				
Pre-drug, rest[b]	73 (10)	71 (11)	1 (-1, 3)	0.18
Post-drug, rest[c]	70 (10)	72 (10)	-2 (-4, 0)	0.07
Change with drug[d]	-3 (11)	-0 (10)	-3 (-6, -1)	0.02
Mean BP (mmHg)				
Pre-drug, rest[b]	92 (16)	89 (11)	3 (1, 5)	0.01
Post-drug, rest[c]	87 (11)	89 (10)	-2 (-5, -0)	0.04
Change with drug[d]	-5 (10)	-1 (9)	-5 (-7, -2)	0.0002

Values represent mean and standard deviation or 25th, 75th percentile values (left columns) and 95% confidence interval for adjusted treatment difference (right column).

[a]Adjusted treatment differences are adjusted for treatment sequence, period effect, a random effect for each participant, and baseline value.

[b]Reflects blood pressure measured at the final visit of study phase, measured prior to administration of allocated study drug for that phase

[c]Reflects blood pressure measured at the final visit of study phase, measured following administration of allocated study drug for that phase

[d]Reflects change from Pre-drug to Post-drug at final visit of study phase

eTable 4. Effects of Open Label Nitrite on Blood Pressure During Run-in

End Point	Pre-Nitrite (n=109)	Post-Nitrite (n=109)	Absolute Treatment Difference	P value
Systolic BP (mmHg)	130 (13)	117 (18)	-12 (-9, -15)	<0.0001
Diastolic BP (mmHg)	72 (10)	66 (11)	-6 (-4, -8)	<0.0001

BP refers to blood pressure

eTable 5. Effects of Nitrite vs Placebo on Additional Exercise End Points

Peak Exercise End Point	Nitrite (n=105)	Placebo (n=105)	Adjusted Treatment Difference[a]	P value
RER[b]	1.1 (1.0, 1.2)	1.1 (1.0, 1.1)	0.01 (-0.01, 0.03)	0.21
Work (Watts)	83 (60, 100)	80 (60, 105)	-2 (-5, 1)	0.26
Systolic BP (mmHg)	154 (136, 180)	156 (136, 174)	-1 (-6, 3)	0.59
Diastolic BP (mmHg)	70 (60, 80)	72 (63, 84)	-3 (-6, -1)	0.0097
Mean BP (mmHg)	97 (89, 109)	101 (90, 110)	-3 (-5, -0)	0.048

[a]Treatment differences are adjusted for treatment sequence, period effect, a random effect for each participant, and baseline value.

[b]RER refers to respiratory exchange ratio defined as the ratio of CO_2 produced to O_2 consumed. Values greater than 1.0 indicate adequate objective effort.

BP refers to systemic blood pressure

eTable 6. Effects of Nitrite vs Placebo on Hours Active per Day

End Point	Nitrite (n=105)	Placebo (n=105)	Adjusted Treatment Difference	P value
Hours active per day	6.2 (5.0, 8.0)	6.5 (4.6, 8.1)	-0.02 (-0.20, 0.17)	0.84

[a]Treatment differences are adjusted for treatment sequence, period effect, and a random effect for each participant.

eAppendix. Detailed Description of End Point Definitions and Methods

Primary endpoint:

Peak VO_2: Cardiopulmonary exercise testing was performed by Core Lab certified sites using equipment and calibration approaches that met American Thoracic Society standards. Cardiopulmonary exercise tests were performed using a 10 Watt/minute incremental ramp protocol and breath-by-breath measures of oxygen uptake were uniformly analyzed by the Core Lab (Massachusetts General Hospital). Peak VO_2 was determined by the highest 30-second median value of breath-by-breath VO_2 measurements during the final minute of incremental exercise performed on an upright cycle ergometer.

Secondary endpoints:

Accelerometry: Participants were supplied with a belt outfitted with two kinetic activity monitors (Kersh Health) containing high-sensitivity, triaxis accelerometers (KXUD9-2050, Kionix) as previously described.[1,2] Patients were instructed to wear the accelerometers 24 hours per day, except while bathing or swimming. The accelerometer measurements are expressed as arbitrary accelerometer units and are stored as 15-minute cumulative accelerometer units (96 data points per 24 hour period). The 15-minute cumulative accelerometer units were totaled over a 14-hour period during the active hours of the day, 7 a.m. to 9 p.m., to provide daily accelerometer units for the secondary endpoint. In addition to averaged daily activity levels, activity levels were assessed comparing the ratio of averaged arbitrary units on study drug to the 2-week period prior to starting study medicine. Accelerometry data represent the mean of values obtained during the 3 weeks on high dose (80 mg) of study drug.

Quality of Life (QOL) Assessment: The KCCQ is a 23-item questionnaire that quantifies multiple QOL domains including physical and social limitations, symptom frequency and severity, overall QOL, recent changes in symptom status, and self-efficacy.[3] The KCCQ is self-administered by patients, and queries respondents to answer based upon the way they have felt over the preceding 2 weeks. The clinical summary scale is the average of physical limitation and total symptoms. The overall summary score is the average of physical limitations, total symptoms, overall QOL, and social limitations. KCCQ scores range from 1 to 100, with higher scores indicative of better QOL.[3,4]

Ventilatory threshold (VT) quantifies VO_2 at the time where anaerobic metabolism is felt to be increasing, determined by the modified V-slope method.[5]

Ventilatory efficiency (V_E/VCO_2 slope) was determined by linear regression of minute ventilation (VE) versus CO2 production (VCO2) from the onset of exercise to peak

exercise, as measured breath-by-breath and averaged every 30 seconds, with exclusion of extreme outliers.[6]

eReferences

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