Supplemental Online Content

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

eTable 1. Allowed chemotherapy regimens (both in the adjuvant and neoadjuvant treatment setting).

Regimen	Drug, dose, rhythm
FEC-D 100	Epirubicin (100 mg/m^2), cyclophosphamide (500 mg/m^2), fluorouracil (500 mg/m^2; every 21 days, for 3 cycles) followed by docetaxel (100 mg/m^2, every 21 days, for 3 cycles)
FEC-D 75	Epirubicin (75 mg/m^2), cyclophosphamide (500 mg/m^2), fluorouracil (500 mg/m^2; every 21 days, for 3 cycles) followed by docetaxel (75 mg/m^2; every 21 days, for 3 cycles)
FEC 100	Epirubicin (100 mg/m^2), cyclophosphamide (500 mg/m^2), fluorouracil (500 mg/m^2; every 21 days, for 6 cycles)
AC	Doxorubicin (60 mg/m^2), cyclophosphamide (600 mg/m^2; every 21 days, for 4 cycles)
EC	Epirubicin (75 mg/m^2), cyclophosphamide (600 mg/m^2; every 21 days, for 4 cycles)
FEC/AC/EC-PAC	FEC or AC or EC (every 21 days) followed by paclitaxel (175 mg/m$^{2;}$ every 21 days, for 4 cycles; or 80 mg/m^2, given weekly for 12 cycles)
EC dose dense	Epirubicin (75 mg/m^2), cyclophosphamide (600 mg/m^2) given every 15 days, for 4 cycles using primary G-CSF prophylaxis given after every cycle.
HER2 positive disease	All anthracycline-containing regimens may be followed by trastuzumab (initial dose: 8 mg/kg; maintenance dose: 6 mg/kg), until completion of 1 year of adjuvant therapy; trastuzumab may be given in combination with paclitaxel or docetaxel.

Abbreviations: HER2, human epidermal growth factor receptor 2.

eTable 2. Patient Characteristics

	Placebo (n = 42)	Ramipril (n = 44)	Bisoprolol (n = 45)	Ramipril - Bisoprolol (n = 43)
Age at diagnosis, years	48.8 ± 9.9	50.6 ± 8.7	48.6 ± 7.9	48.1 ± 8.9
Stage				
I	14 (33)	16 (36)	13 (29)	10 (23)
II	20 (48)	21 (48)	24 (53)	25 (58)
III	8 (19)	7 (16)	8 (18)	8 (19)
HR positive	36 (86)	29 (66)	37 (82)	30 (70)
Surgery				
Breast Conserving Surgery	21 (50)	23 (52)	23 (51)	19 (44)
Mastectomy	21 (50)	21 (48)	22 (49)	24 (56)
Height, cm	163.7 ± 5.5	163.8 ± 6.5	161.9 ± 6.7	165.8 ± 5.5
Weight, kg	64.5 ± 11.1	62.2 ± 11.2	63.1 ± 10.9	65.1 ± 11.2
BSA, m²	1.70 ± .14	1.67 ± .15	1.67 ± .14	1.72 ± .14
Systolic blood pressure, mmHg	120.8 ± 11.9	125.2 ± 13.7	122.6 ± 11.3	124.3 ± 11.3
Diastolic blood pressure, mmHg	72.6 ± 8.7	73.8 ± 10.3	72.7 ± 8.5	75.6 ± 9.0
Heart rate, bpm	72.9 ± 10.4	72.6 ± 12.9	71.5 ± 9.7	74.5 ± 11.1
Hemoglobin, g/dL	13.0 ± 1.0	13.3 ± 1.3	13.1 ± 1.3	13.2 ± 1.1
NT proBNP, pg/mL	78.7 ± 69.2	71.6 ± 58.7	71.3 ± 49.4	56.7 ± 36.8
Body mass index	24.1 ± 4.3	23.2 ± 4.0	24.1 ± 4.2	23.7 ± 4.0
Current smokers	6 (13)	7 (16)	1 (2)	3 (7)
Ex-smokers	4	4	3	6
No smokers	35	32	38	35
Hypertension	1 (2)	3 (7)	0	2 (5)
Troponin (>95% CI)	1 (2)	0	0	0
Neoadjuvant chemotherapy	11 (26)	10 (23)	8 (18)	11 (26)
Chemotherapy regimen				
AC/EC-PAC	39 (93)	41 (93)	41 (91)	39 (91)
FEC-D	0	2 (5)	3 (7)	1 (2)
FEC-100	2 (5)	0	1 (2)	2 (5)
AC/EC	1 (2)	1 (2)	0	1 (2)
Additional therapy				
Trastuzumab	18 (43)	15 (34)	15 (33)	16 (37)
Radiation therapy	26 (62)	22 (50)	27 (60)	26 (60)

NOTE. Data are reported as mean ± standard deviation (SD) or n (%). No significant differences were found in any group distribution.

Abbreviations: SD, standard deviation; HR, hormonal receptors; BSA, body surface area; NT proBNP, N-Terminal Pro-Brain Natriuretic Peptide; 95% CI, 95% confidence interval; AC/EC-PAC, adriamycin or epirubicin in combination with cyclophosphamide followed by weekly paclitaxel; FEC-D, fluorouracil, epirubicin, cyclophosphamide followed by docetaxel

eTable 3. Echocardiography imaging measures and study drug hemodynamic effect stratified by treatment arm at each time-point (ITT analysis).

Measure	**Arm**	**EOT**, n	**Baseline**	**3-month**	**6-month**	**12-month**	***P*°**
3D-LVEF, %	Placebo	42	67.3 ± 4.2	64.9 ± 4.7[d]	63.5 ± 4.1[d]	63.9 ± 3.7[d]	
	Ramipril	44	65.7 ± 3.4	64.9 ± 3.0	64.4 ± 3.7	64.2 ± 3.1	
	Bisoprolol	45	66.5 ± 4.1	65.5 ± 4.2	65.1 ± 3.5	65.2 ± 4.1	
	Ramipril - Bisoprolol	43	66.4 ± 3.1	65.9 ± 3.5	65.0 ± 3.8	65.6 ± 3.9	.015
GLS	Placebo	42	-24.1 ± 1.9	-22.9 ± 2.1[d]	-22.3 ± 2.1[d]	-22.3 ± 2.0[d]	
	Ramipril	44	-23.1 ± 1.5	-22.7 ± 1.5	-22.6 ± 1.7	-22.9 ± 1.9	
	Bisoprolol	45	-23.3 ± 2.2	-23.0 ± 2.1	-23.0 ± 2.2	-23.2 ± 2.0	
	Ramipril - Bisoprolol	43	-23.0 ± 1.9	-23.3 ± 2.1	-22.9 ± 2.0	-23.3 ± 1.9	.0001
EDVI, mL/m^2	Placebo	42	52.4 ± 7.7	56.4 ± 8.9[d]	57.7 ± 8.9[d]	58.4 ± 9.9[d]	
	Ramipril	44	52.3 ± 8.9	54.4 ± 9.1[b]	54.7 ± 9.5	54.0 ± 9.9	
	Bisoprolol	45	53.5 ± 9.9	53.4 ± 9.4	53.2 ± 8.9	53.9 ± 8.9	
	Ramipril - Bisoprolol	43	52.3 ± 6.5	51.9 ± 6.0	52.4 ± 7.2	52.3 ± 6.3	.0001
ESVI, mL/m^2	Placebo	42	17.2 ± 3.7	19.8 ± 4.0[d]	21.2 ± 4.7[d]	21.3 ± 5.6[d]	
	Ramipril	44	18.0 ± 3.7	19.1 ± 3.9[c]	19.5 ± 4.3[c]	19.4 ± 4.4[a]	
	Bisoprolol	45	17.9 ± 3.9	18.4 ± 3.7	18.6 ± 3.7	18.8 ± 4.0	
	Ramipril - Bisoprolol	43	17.6 ± 2.8	17.7 ± 2.8	18.3 ± 3.0[a]	18.0 ± 2.7	.0001
E/A	Placebo	42	1.2 ± .3	1.1 ± .3	1.0 ± .2[a]	1.1 ± .3	
	Ramipril	44	1.2 ± .3	1.2 ± .3	1.1 ± .3	1.2 ± .4	
	Bisoprolol	45	1.2 ± .3	1.4 ± .4[c]	1.2 ± .4	1.3 ± .4[a]	
	Ramipril - Bisoprolol	43	1.2 ± .3	1.3 ± .4	1.2 ± .3	1.3 ± .4	.005
E/e'	Placebo	42	7.5 ± 1.7	7.7 ± 1.7	8.1 ± 1.7	7.9 ± 1.9	
	Ramipril	44	7.2 ± 1.3	7.1 ± 1.3	7.6 ± 1.6	7.6 ± 1.6	
	Bisoprolol	45	7.6 ± 1.8	8.2 ± 1.8[a]	8.2 ± 1.8	8.2 ± 1.9	
	Ramipril - Bisoprolol	43	7.2 ± 1.5	7.5 ± 1.4	7.6 ± 1.7	7.8 ± 1.7	.531
LAV, mL/m^2	Placebo	42	24.5 ± 5.6	25.0 ± 6.1	26.8 ± 5.9[d]	24.4 ± 5.7	
	Ramipril	44	24.1 ± 4.8	24.9 ± 6.4	24.3 ± 6.0	22.2 ± 5.6	
	Bisoprolol	45	23.0 ± 5.5	27.5 ± 5.1[d]	26.9 ± 5.4[d]	24.8 ± 7.3	
	Ramipril - Bisoprolol	43	22.7 ± 4.6	24.9 ± 6.2	24.2 ± 6.4	23.7 ± 5.0	.119
SAP, mmHg	Placebo	42	122.6 ± 11.3	117.5 ± 15.4	122.5 ± 11.2	121.7 ± 14.9	
	Ramipril	44	124.3 ± 11.3	117.3 ± 10.6[d]	117.5 ± 10.5[d]	119.4 ± 12.7[a]	
	Bisoprolol	45	120.8 ± 11.9	113.6 ± 14.5	116.6 ± 14.3	118.2 ± 15.4	
	Ramipril - Bisoprolol	43	125.2 ± 13.7	113.7 ± 13.2[d]	112.2 ± 14.8[d]	116.3 ± 13.7[d]	.001

DAP, mmHg	Placebo	42	72.7 ± 8.5	71.0 ± 11.0	74.5 ± 10.1	73.2 ± 10.3	
	Ramipril	44	75.6 ± 9.0	71.3 ± 8.7[c]	72.9 ± 8.8	75.9 ± 13.6	
	Bisoprolol	45	72.6 ± 8.7	69.1 ± 8.9[a]	70.5 ± 8.8	70.1 ± 10.2	
	Ramipril - Bisoprolol	43	73.8 ± 10.3	67.0 ± 10.2d	68.7 ± 9.5[d]	70.4 ± 9.4	.021
HR, bpm	Placebo	42	71.5 ± 9.7	79.2 ± 11.1[d]	80.8 ± 13.3[d]	71.4 ± 12.0	
	Ramipril	44	74.5 ± 11.1	80.1 ± 12.9[d]	79.6 ± 12.5[a]	70.9 ± 10.8	
	Bisoprolol	45	72.9 ± 10.4	65.0 ± 9.4[d]	66.0 ± 8.2[d]	60.6 ± 7.5[d]	
	Ramipril - Bisoprolol	43	72.6 ± 12.9	66.8 ± 12.3[a]	68.7 ± 12.2	64.7 ± 12.8[c]	.0001

NOTE. Data are reported as means ± standard deviation (SD).

Abbreviations: EOT, end of allocated treatment; 3D-LVEF, 3-dimensional left ventricular ejection fraction; GLS, global longitudinal strain; EDVI, indexed left ventricular end diastolic volume; ESVI, indexed left ventricular end systolic volume; E/A, early to late diastolic transmitral flow velocity ratio; E/e', early diastolic transmitral flow velocity to early diastolic mitral annular tissue velocity ratio; LAV, left atrial volume; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR, heart rate.

° General linear modeling repeated measures, Helmet contrast, *P* value form by Greenhouse-Geisser correction.
[a] *P* < .05 compared with baseline.
[b] *P* < .01 compared with baseline.
[c] *P* < .005 compared with baseline.
[d] *P* < .001 compared with baseline.

eTable 4. Echocardiography imaging measures and study drug hemodynamic effect comparison between baseline and end of treatment (ITT analysis).

	n	Baseline mean (95%CI) unadjusted	EOT mean (95% CI) unadjusted	EOT mean (95% CI) adjusted	% changes from baseline to EOT	*P*[a]	*P*[b]
3D-LVEF							
No ramipril	87	66.8 (66.0, 67.7)	64.6 (63.7, 65.4)	64.4 (63.7, 65.1)	-3.1	.213	.071
Ramipril	87	66.1 (65.4, 66.8)	64.9 (64.1, 65.6)	65.0 (64.3, 65.8)	-2.1		
No bisoprolol	86	66.5 (65.6, 67.3)	64.0 (63.3, 64.8)	64.0 (63.3, 64.8)	-3.6	.008[c]	.024[c]
Bisoprolol	88	66.5 (65.7, 67.2)	65.4 (64.6, 66.2)	65.4 (64.7, 66.1)	-1.6		
All	174	66.5 (65.9, 67.0)					
GLS							
No ramipril	87	-23.7 (-24.1, -23.2)	-22.8 (-23.2, -22.3)	-22.6 (-23.0, -22.2)	-3.2	.045[c]	.004[c]
Ramipril	87	-23.1 (-23.4, -22.7)	-23.1 (-23.5, -22.7)	-23.2 (-23.6, -22.8)	-0.7		
No bisoprolol	86	-23.6 (-23.9, -23.2)	-22.6 (-23.0, -22.2)	-22.6 (-22.9, -22.2)	-3.5	.006[c]	.002[c]
Bisoprolol	88	-23.2 (-23.6, -22.7)	-23.3 (-23.7, -22.8)	-23.3 (-23.7, -22.9)	-0.1		
All	174	-23.4 (-23.6, -23.1)					
EDVI							
No ramipril	87	53.0 (51.1, 54.9)	56.1 (54.0, 58.1)	55.7 (54.6, 56.8)	5.9	.006[c]	.008[c]
Ramipril	87	52.3 (50.6, 54.0)	53.2 (51.4, 55.0)	53.5 (52.4, 54.6)	1.7		
No bisoprolol	86	52.4 (50.6, 54.2)	56.2 (54.0, 58.3)	56.4 (55.4, 57.5)	7.2	<.0001[c]	<.0001[c]
Bisoprolol	88	52.9 (51.1, 54.7)	53.1 (51.5, 54.8)	52.9 (51.9, 54.0)	0.5		
All	174	52.7 (51.4, 53.9)					
ESVI							
No ramipril	87	17.6 (16.8, 18.4)	20.0 (18.9, 21.0)	20.0 (19.4, 20.7)	13.5	.004[c]	.004[c]
Ramipril	87	17.8 (17.1, 18.5)	18.7 (17.9, 19.5)	18.6 (17.9, 19.3)	5.4		
No bisoprolol	86	17.6 (16.8, 18.4)	20.3 (19.2, 21.4)	20.4 (19.7, 21.0)	15.3	<.0001[c]	<.0001[c]
Bisoprolol	88	17.7 (17.0, 18.5)	18.4 (17.7, 19.1)	18.3 (17.7, 19.0)	3.7		
All	174	17.7 (17.1, 18.2)					
E/A							
No ramipril	87	1.2 (1.1, 1.2)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	3.6	.421	.442
Ramipril	87	1.2 (1.1, 1.2)	1.3 (1.2, 1.3)	1.3 (1.2, 1.3)	7.2		
No bisoprolol	86	1.2 (1.1, 1.2)	1.1 (1.1, 1.2)	1.1 (1.1, 1.2)	-3.2	<.0001[c]	<.0001[c]
Bisoprolol	88	1.2 (1.1, 1.2)	1.3 (1.2, 1.4)	1.3 (1.3, 1.4)	13.7		
All	174	1,2 (1.1, 1.2)					
E/e'							
No ramipril	87	7.5 (7.1, 7.9)	8.1 (7.7, 8.5)	8.0 (7.6, 8.3)	8.3	.492	.987
Ramipril	87	7.2 (6.9, 7.5)	7.7 (7.4, 8.1)	7.8 (7.5, 8.1)	6.2		
No bisoprolol	86	7.3 (7.0, 7.6)	7.8 (7.4, 8.1)	7.8 (7.5, 8.1)	6.1	.377	.459

Bisoprolol	88	7.4 (7.0, 7.7)	8.0 (7.6, 8.4)	8.0 (7.7, 8.3)	8.8		
All	174	7.3 (7.1, 7.6)					
LAV							
No ramipril	87	23.7 (22.5, 24.9)	24.6 (23.2, 26.0)	24.5 (23.3, 25.8)	4.2	.083	.208
Ramipril	87	23.4 (22.4, 24.4)	23.0 (21.8, 24.1)	23.0 (21.8, 24.2)	-2.4		
No bisoprolol	86	24.3 (23.1, 25.4)	23.3 (22.0, 24.5)	23.2 (21.9, 24.4)	-1.6	.12	.025[c]
Bisoprolol	88	22.9 (21.8, 24.0)	24.2 (22.9, 25.6)	24.6 (23.3, 25.8)	4.3		
All	174	23.6 (22.8, 24.3)					
SAP							
No ramipril	87	121.6 (119.2, 124.1)	119.9 (116.7, 123.2	121.3 (118.9, 123.7)	-1.6	.012[c]	.004[c]
Ramipril	87	124.8 (122.1, 127.4)	117.9 (115.1, 120.7)	116.9 (114.5, 119.3)	-5.1		
No bisoprolol	86	123.5 (121.1, 125.9)	120.5 (117.6, 123.5)	120.3 (117.9, 122.7)	-2.4	.103	.142
Bisoprolol	88	122.9 (120.2, 125.7)	117.3 (114.2, 120.4)	117.5 (115.1, 119.9)	-4.6		
All	174	123.2 (121.4, 125.0)					
DAP							
No ramipril	87	72.6 (70.8, 74.4)	71.6 (69.4, 73.8)	72.4 (70.5, 74.4)	-1.7	.889	.771
Ramipril	87	74.7 (72.6, 76.7)	73.2 (70.7, 75.8)	72.6 (70.7, 74.6)	-1.4		
No bisoprolol	86	74.2 (72.3, 76.0)	74.6 (72.0, 77.2)	74.2 (72.3, 76.1)	0.7	.009°	.021[c]
Bisoprolol	88	73.2 (71.2, 75.2)	70.3 (68.2, 72.3)	70.5 (68.6, 72.5)	-4.2		
All	174	73.7 (72.3, 75.0)					
HR							
No ramipril	87	72.2 (70.1, 74.4)	65.9 (63.5, 68.3)	66.1 (63.8, 68.4)	-9.3	0.387	.682
Ramipril	87	73.5 (71.0, 76.1)	67.8 (65.2, 70.4)	67.5 (65.3, 69.7)	-7.4		
No bisoprolol	86	73.0 (70.8, 75.3)	71.2 (68.7, 73.6)	71.1 (69.0, 73.2)	-2.4	<0.0001[c]	<.0001[c]
Bisoprolol	88	72.7 (70.3, 75.2)	62.6 (60.4, 64.9)	62.7 (60.6, 64.7)	-14.0		
All	174	72.9 (71.2, 74.5)					

NOTE. Data are reported as means (95% CI).

Abbreviations: EOT, end of allocated treatment; 3D-LVEF, 3-dimensional left ventricular ejection fraction; GLS, global longitudinal strain; EDVI, indexed left ventricular end diastolic volume; ESVI, indexed left ventricular end systolic volume; E/A, early to late diastolic transmitral flow velocity ratio; E/e', early diastolic transmitral flow velocity to early diastolic mitral annular tissue velocity ratio; LAV, left atrial volume; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR, heart rate.

[a] Statistical analysis was performed by ANCOVA, covariates were compared by least significant difference vs. no drug (ramipril or bisoprolol).
[b] Statistical analysis was performed by general linear modeling repeated measures, Helmet contrast, p value form by Greenhouse-Geisser correction.
[c] $P < .05$.

eTable 5. Echocardiography imaging measures and study drug hemodynamic effect comparison between baseline, end of treatment, end of study observation (ITT analysis).

	n	T0 mean (95% CI) baseline unadjusted	EOT mean (95% CI) 12-month adjusted	% changes from baseline to EOT	*P*[a]	*P*[b]	EOS mean (95% CI) 24-month adjusted	% changes from EOT to EOS	% changes from T0 to EOS	*P*[a]	*P*[b]
LVEF 3D, %											
No ramipril	64	67.3 (66.3, 68.4)	65.1 (64.2, 65.9)	-3.0	.834	.470	64.5 (63.8, 65.3)	0.8	-3.8	.617	.343
Ramipril	61	66.7 (66.0, 67.5)	65.2 (64.3, 66.1)	-2.8			64.8 (64.0, 65.6)	0.6	-3.4		
No bisoprolol	62	67.1 (66.0, 68.1)	64.1 (63.3, 65.0)	-4.3	.001[c]	.006[c]	64.2(63.4, 64.9)	-0.1	-4.3	.062	.117
Bisoprolol	63	67.0 (66.2, 67.9)	66.1 (65.3, 67.0)	-1.4			65.2 (64.4, 65.9)	1.5	-2.8		
All	125	67.1 (66.4, 67.7)									
GLS											
No ramipril	64	-24.1 (-24.6, -23.6)	-22.6 (-23.1, -22.1)	-4.5	.111	.008[c]	-22.8 (-23.3, -22.4)	-0.9	-3.7	.051	.004[c]
Ramipril	61	-23.3 (-23.7, -22.9)	-23.2 (-23.7, -22.7)	-2.1			-23.4 (-23.9, -23.0)	-1.0	-1.1		
No bisoprolol	62	-23.8 (-24.3, -23.4)	-22.4 (-22.9, -22.0)	-5.3	.003[c]	.004[c]	-22.9 (-23.3, -22.5)	-2.0	-3.4	.067	.051
Bisoprolol	63	-23.6 (-24.1, -23.1)	-23.5 (-24.0, -23.0)	-0.8			-23.5 (-23.9, -23.0)	0.2	-1.0		
All	125	-23.7(-24.0, -23.4)									
EDVI, mL/m^2											
No ramipril	64	54.5 (52.3, 56.6)	55.5 (54.2, 56.9)	4.7	.022[c]	.031[c]	55.3 (53.8, 56.8)	0.5	4.3	.019	.034[c]
Ramipril	61	51.5 (49.7, 53.3)	53.2 (51.8, 54.6)	0.4			52.7 (51.2, 54.2)	1.0	-0.6		
No bisoprolol	62	52.5 (50.6, 54.5)	57.1 (55.9, 58.3)	7.7	.0001[c]	.0001[c]	56.3 (54.9, 57.7)	1.4	6.2	.0001[c]	.0001[c]
Bisoprolol	63	53.5 (51.4, 55.6)	51.9 (50.7, 53.1)	-2.1			52.1 (50.7, 53.5)	-0.4	-1.7		
All	125	53.0 (51.6, 54.4)									
ESVI, mL/m^2											
No ramipril	64	17.8 (16.8, 18.7)	19.7 (18.8, 20.6)	12.6	.039[a]	.052	19.8 (19.0, 20.7)	-0.8	13.5	.016[c]	.029[c]
Ramipril	61	17.2 (16.4, 17.9)	18.4 (17.5, 19.3)	5.1			18.4 (17.5, 19.2)	-0.1	5.2		
No bisoprolol	62	17.3 (16.4, 18.2)	20.6 (19.8, 21.4)	17.7	.0001[c]	.0001[c]	20.2 (19.4, 21.0)	1.6	15.8	.0001[c]	.0001[c]
Bisoprolol	63	17.6 (16.8, 18.4)	17.6 (16.8, 18.4)	0.8			18.1 (17.3, 18.9)	-3.0	3.8		
All	125	17.5 (16.9, 18.1)									
E/A											
No ramipril	64	1.2 (1.1, -1.3)	1.2 (1.1, 1.3)	2.9	.466	.483	1.1 (1.1, 1.2)	6.4	-3.7	.661	.655
Ramipril	61	1.2 (1.1, -1.3)	1.3 (1.2, 1.4)	6.8			1.1 (1.0, 1.2)	11.8	-5.8		
No bisoprolol	62	1.2 (1.1, -1.3)	1.1 (1.0, 1.2)	-4.2	.001[c]	.001[c]	1.1 (1.1, 1.2)	0.0	-4.2	.055	.832
Bisoprolol	63	1.2 (1.1, -1.3)	1.3 (1.3, 1.4)	13.7			1.1 (1.0, 1.2)	16.6	-5.2		
All	125	1.2 (1.1, 1.2)									
E/e'											
No ramipril	64	7.4 (6.9, 7.9)	7.9 (7.5, 8.3)	7.8	.378	.656	7.7 (7.3, 8.0)	2.9	4.6	.694	.514

Ramipril	61	7.2 (6.9, 7.6)	7.7 (7.3, 8.0)	4.6			7.8 (7.4, 8.2)	-1.5	6.1		
No bisoprolol	62	7.3 (6.9, 7.6)	7.7 (7.3, 8.1)	5.2	.523	.686	7.8 (7.4, 8.2)	-1.2	6.4	.621	.542
Bisoprolol	63	7.4 (6.9, 7.8)	7.9 (7.5, 8.2)	7.5			7.6 (7.3, 8.0)	2.8	4.5		
All	125	7.3 (7.0, 7.6)									
LAV, mL/m2											
No ramipril	64	24.2 (22.7, 25.6)	25.9 (24.5, 27.4)	10.0	.061	.425	19.4 (18.0, 20.7)	25.4	-17.9	.518	.184
Ramipril	61	22.9 (21.8, 24.1)	24.0 (22.5, 25.4)	1.6			20.0 (18.6, 21.3)	16.6	-15.3		
No bisoprolol	62	24.2 (22.8, 25.5)	24.4 (22.9, 25.8)	3.4	.208	.083	19.5 (18.2, 20.8)	20.0	-17.3	.425	.176
Bisoprolol	63	23.0 (21.7, 24.3)	25.7 (24.2, 27.1)	9.0			20.2 (18.9, 21.6)	21.2	-14.1		
All	125	23.6 (22.6, 24.5)									
SAP, mmHg											
No ramipril	64	122 (119, 125)	121 (119, 124)	-1.5	.031[c]	.021[c]	122 (120,125)	-0.6	-0.9	.156	.087
Ramipril	61	124 (121, 127)	117 (114,120)	-5.1			120 (117,122)	-2.2	-3.0		
No bisoprolol	62	123 (120, 126)	121 (118,123)	-2.1	.115	.120	120 (118,123)	0.3	-2.4	.666	.687
Bisoprolol	63	123 (120, 126)	117 (114,120)	-4.8			121 (119,124)	-3.2	-1.7		
All	125	123 (121, 125)									
DAP, mmHg											
No ramipril	64	71.6 (69.5, 73.7)	71.3 (68.8, 73.7)	-1.8	.624	.849	75.0 (72.7, 77.3)	-5.2	3.3	.934	.734
Ramipril	61	73.6 (71.3, 75.9)	72.1 (69.6, 74.6)	-0.6			75.2 (72.8, 77.5)	-4.2	3.5		
No bisoprolol	62	73.0 (70.9, 75.1)	74.2 (71.9, 76.6)	2.2	.002[c]	.005[c]	75.1 (72.8, 77.5)	-1.2	3.5	.834	.979
Bisoprolol	63	72.2 (69.9, 74.5)	69.0 (66.6, 71.4)	-5.0			74.8 (72.5, 77.1)	-8.4	3.0		
All	125	72.6 (71.1, 74.1)									
HR, bpm											
No ramipril	64	73.4 (70.7, 76.1)	67.1 (64.3, 69.8)	-9.2	0.639	.889	68.4 (65.9, 70.8)	-1.9	-7.5	.158	.321
Ramipril	61	74.4 (71.5, 77.3)	68.0 (65.2, 70.8)	-7.9			70.9 (68.4, 73.3)	-4.2	-4.1		
No bisoprolol	62	73.7 (71.0, 76.4)	72.5 (70.0, 75.0)	-1.9	.0001[c]	.0001[c]	70.7 (68.2, 73.3)	2.4	-4.2	.223	.242
Bisoprolol	63	74.1 (71.2, 77.0)	62.8 (60.3, 65.2)	-15.0			68.6 (66.1, 71.0)	-9.2	-7.2		
All	125	73.9 (71.9, 75.8)									

NOTE. Data are reported as means (95% CI).

Abbreviations: EOT, end of allocated treatment; EOS, end of study observation; 3D-LVEF, 3-dimensional left ventricular ejection fraction; GLS, global longitudinal strain; EDVI, indexed left ventricular end diastolic volume; ESVI, indexed left ventricular end systolic volume; E/A, early to late diastolic transmitral flow velocity ratio; E/e', early diastolic transmitral flow velocity to early diastolic mitral annular tissue velocity ratio; LAV, left atrial volume; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR, heart rate.

[a] Statistical analysis was performed by ANCOVA, covariates were compared by least significant difference vs. no drug (ramipril or bisoprolol).
[b] Statistical analysis was performed by general linear modeling repeated measures, Helmet contrast, p value form by Greenhouse-Geisser correction
[c] $P < .05$.

eTable 6. Study drug dose titration and tolerability.

	Placebo (n = 42)	Ramipril (n = 44)	Bisoprolol (n = 45)	Ramipril - Bisoprolol (n = 43)
Dose titration, n (%)				
100% dose[a]	38 (90.4)	43 (97.7)	43 (95.4)	34 (79.1)[b]
50% dose	3 (7.2)	0	1 (2.3)	7 (16.3)
25% dose	0	0	0	1 (2.3)
Discontinuation	1 (2.4)	1 (2.3)	1 (2.3)	1 (2.3)
Dose reduction/discontinuation cause, n (%)				
Hypotension	3 (7.2)	0	1 (2.3)	6 (14)[b]
Cough	0	1 (2.3)	0	2 (4.7)[b]
Bradycardia	0	0	1 (2.3)	1 (2.3)
Heart Failure	1 (2.4)	0	0	0

[a] 100% dose = bisoprolol and/or ramipril 5 mg daily.
[b] $P < 0.05$ compared with other groups.

eMethods 1. Main inclusion and exclusion criteria.

Trial inclusion criteria were the following: non-metastatic, histology-proven, BC; indication to primary or postoperative systemic therapy using an anthracycline-based regimen with or without trastuzumab; written informed consent; age ≥18 years.
Exclusion criteria were previous treatment with anthracyclines; ongoing treatment with ACEi/Angiotensin-II Receptor Blockers or BB; baseline LVEF <50%; previous diagnosis of solid tumors treated with systemic therapy; recurrent and/or metastatic BC; impossibility of LV function evaluation at echocardiography; heart failure symptoms; prior diagnosis of cardiomyopathy, coronary artery disease, moderate to severe mitral and aortic disease; pharmacological therapy for asthma.

eMethods 2. Cardiac assessment.

Medical history, electrocardiogram (EKG), clinical examination with specific attention to signs of heart failure, NYHA class and Canadian angina grading scale score were recorded during each visit. Transthoracic echocardiography was performed with a commercially available system (EPIQ, X5-1 transducer, Philips Healthcare, Andover, Massachusetts). All measurements were performed and reported accordingly to the American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI) recommendations [1]. Each measure was averaged over three cardiac cycles. Two-dimensional LVEF (2D-LVEF) was measured by Simpson rule, throughout apical 4- and 2-chamber views. LV mass was calculated from LV oriented M-mode tracings using the Devereux formula [2], ASE convention [1], and was indexed to body surface area (BSA) [3]. Diastolic function was evaluated by left atrial volume index, systolic pulmonary pressure, mitral inflow E/A pattern, E/A ratio, E velocity deceleration time, annular tissue Doppler e', and E/e' ratio [4]. After optimizing image quality, maximizing frame rate, and minimizing foreshortening, which are all critical to reduce measurement variability, GLS measurements were made in the three standard apical views and averaged. LV 4-, 3-, and 2-chamber views were acquired during breath hold. From apical long-axis view to visualize aortic valve closure, using opening and closing clicks of the aortic valve the timing of aortic valve opening and closing on continuous wave (CW) Doppler imaging respect to EKG R wave was measured. Full-volume six-cycles gated acquisition breath hold images of left ventricle were acquired for Q-lab analysis to obtain end-diastolic volume, end systolic volume, indexed to BSA (EDVI and ESVI) and 3D-LVEF [5]. Q-lab version in this study was 10.5. All echocardiography data was stored including the original Digital Imaging and Communications in Medicine (DICOM) images. All scans were read jointly by two experienced board certified echocardiographers who were blinded to all clinical characteristics. Systemic arterial pressure was measured simultaneously with echo measurements, by means of an arm-cuff sphygmomanometer. EKG was performed using ELI 200 Mortara electrocardiograph. The heart rate and QTc measurement according to Fridericia correction were recorded.

eMethods 3. Statistical Methods.

The primary endpoint was defined as detection of any subclinical impairment in myocardial function and deformation (worsening ≥10%) measured with standard and 3-dimensional (3D) echocardiography and left ventricular (LV) global longitudinal strain (GLS). This is a pre-specified interim analysis on the first 174 patients who had completed cardiological assessment at 12-month (T3) and reached the EOT.
An independent data and safety monitoring committee was established to advise the investigators at regular intervals and potential need for premature study termination due to safety, efficacy or slower than anticipated recruitment. Although the interim analysis results were significantly in favor of a cardioprotective strategy, due to the slow rate of enrollment, worsened by the outbreak of COVID-19 that has severely affected the ability to safely conduct clinical trials, the study promoter decided to prematurely terminate recruitment in June 2020 [6-8]. An updated final analysis is already pre-planned when all the 262 enrolled patients will reach the EOS (June 2022).
Patients were allocated in a 2x2 factorial design to one of the four treatment arms **(Figure 1)**. Allocation of the participants in the trial arms was conducted by a stratified randomization, using permuted blocks within defined age and HER2 status strata, software based. The reported rate of subclinical cardiotoxicity measured with traditional methods and tissue Doppler imaging varies in a range of 20–75% [9-13]. Considering a median rate of subclinical alteration of 40%, with 15% reduction in treatment groups to reach the outcome, the study design required a sample of 90 patients per treatment group provided an 80% statistical power. The interim analysis was based to test an anticipated 10% change in 3D-LVEF, two-tailed significance level of a = .05, 80% power, 43 patients per group required. This analysis statistics is also supported by the MANTICORE trial assumptions [14], where considering an anticipated 11% change in EDVI within-group, a two-tailed significance level of a = .05, and 80% power required 47 patients per group.
To compare the individual characteristics of patients allocated in the distinct groups at T0 (baseline), Chi-squared test or Fisher exact test for categorical variables and ANOVA and REGW-Q for continuous variables are used. Primary endpoint was evaluation of reduction in both systolic and diastolic, early and late subclinical cardiotoxicities measured with standard cardiac echo-color Doppler, 3D echocardiography and LV GLS in pharmacologically treated patients as compared to the placebo group. All references to GLS changes actually consider the absolute value of the number [15]. For each continuous efficacy endpoint, we analyzed the subgroups by repeated measure linear mixed model and for each subgroup by ANOVA and t test with Bonferroni correction. We also performed analysis by ANCOVA, covariates were compared by least significant difference vs. no drug (ramipril or bisoprolol). We fitted a linear mixed model to all available measurements from three time points: (i) baseline, (ii) EOT either after completion of the final cycle of anthracycline therapy and the completion of trastuzumab, and (iii) the completion of 2 years' study (EOS). To investigate possible interactions between the two treatments, we fitted additional models that included a ramipril-per-bisoprolol interaction term and applied a likelihood ratio test to the models with and without the treatment interaction term. No statistically significant treatment interactions were observed. The treatment effects were estimated for patients in four groups: (i) treated with ramipril, (ii) not treated with ramipril, (iii) treated with bisoprolol, and (iv) not treated with bisoprolol using both the between-group difference in change from baseline to EOT and EOS for the comparisons of ramipril vs. no ramipril treatment and bisoprolol vs. no bisoprolol treatment by general linear model with repeated measures and Greenhouse-Geisser correction of within-subject effects and by ANCOVA. The analysis was based on intention to treat (ITT) population. Statistical analyses were performed using IBM SPSS version 26.0 (Armonk, NY: IBM Corp).

Supplement References

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