

SUPPLEMENTAL MATERIAL

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): Extended follow-up of a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol

Contents

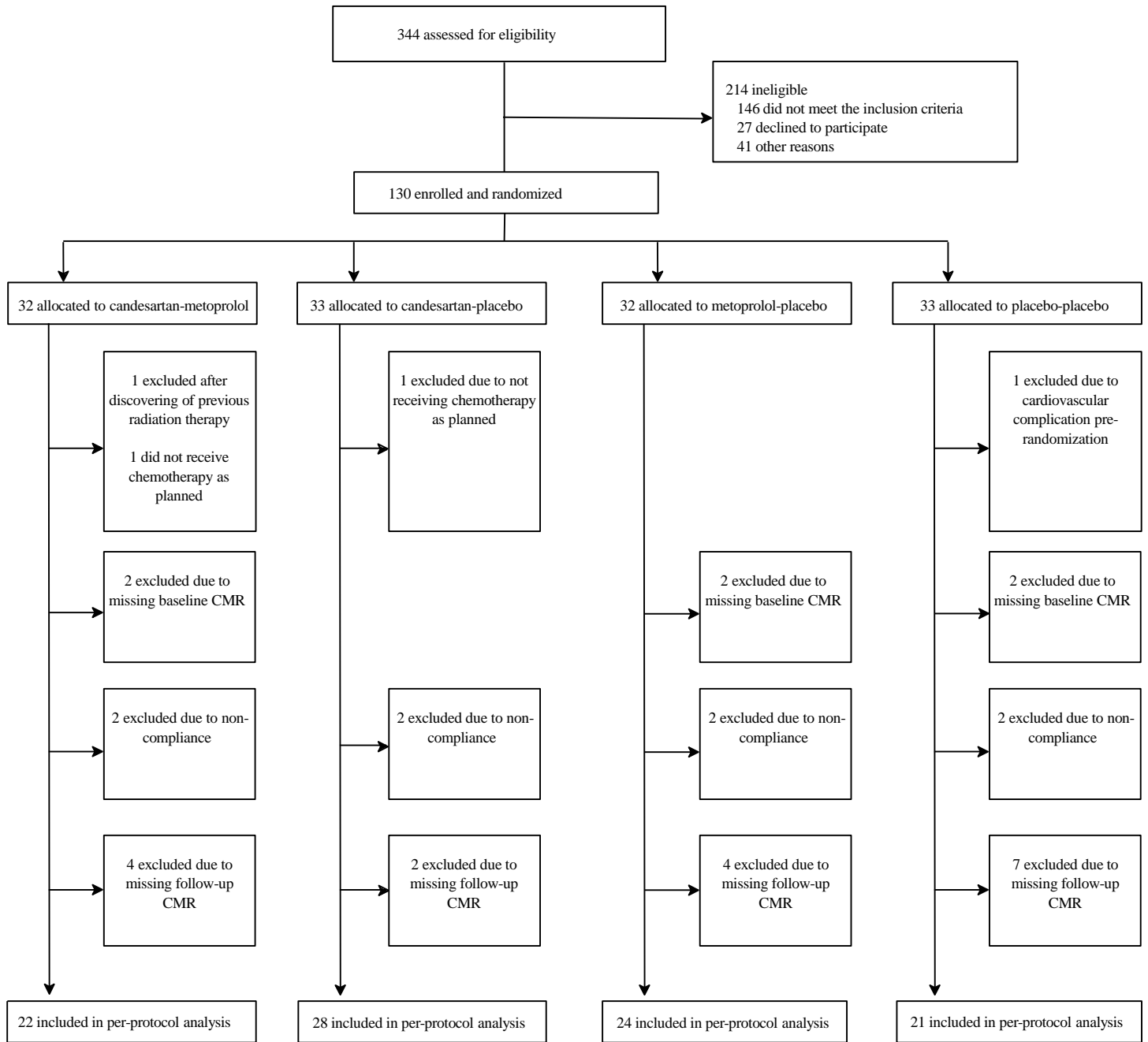
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Blood Sampling and Biochemical Analysis

On follow-up, non-fasting samples of venous blood were drawn, put on ice and processed within 60 minutes. EDTA-plasma specimens were centrifuged at 13 500 and serum specimens at 3500 relative centrifugal force for 30 minutes; the clear supernatants were then transferred to the sample cups and stored at -80°C. Before analysis, thawed specimens were mixed thoroughly by low-speed vortexing until visibly homogeneity.

Serum cardiac troponin I (cTnI) on follow-up was measured by STAT High Sensitive Troponin-I assay on an Alinity platform (Abbott Diagnostics, Abbott, IL). The level of blank was 1.0 ng/L and the level of detection 1.6 ng/L. Concentrations below or equal to the level of blank were assigned a value of 1.0, whereas levels below or equal to the level of detection and greater than the level of blank, were assigned a value of 1.6 ng/L. At baseline and during adjuvant therapy, cTnI was measured by STAT High Sensitive Troponin-I assay on an Architect i2000SR platform (Abbott Diagnostics, Abbott, IL). For this assay level of blank 0.8 ng/L and the level of detection 1.2 ng/L, concentrations below or equal to the limit of blank were assigned a value of 0.8 ng/L, whereas levels below or equal to the limit of detection and greater than the limit of blank were assigned a value of 1.2 ng/L.

Serum cTnT on follow-up was measured by a high-sensitivity assay (Troponin T hs STAT) on a cobas 8000 e602 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The level of blank was 2.5 ng/L and the level of detection 3 ng/L. Values equal to or below 3.0 ng/L were assigned a value of 3.0. At baseline and during adjuvant therapy, cTnT was measured with cobas 8000 e602 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The level of blank was 3.0 ng/L and the level of detection 5 ng/L. Concentrations below or equal to the limit of blank were assigned a value of 3.0 ng/L, whereas levels below or equal to the limit of detection and greater than the limit of blank were assigned a value of 5 ng/L. N-terminal pro B-type natriuretic peptide (NT-proBNP) in serum was on follow-up measured by the proBNP II assay on a cobas 8000 e801 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The analytical measurement range is 5 to 35 000 pg/mL. At baseline and during adjuvant therapy, NT-proBNP was measured with cobas 8000 e602 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) where the analytical range was 5 to 35 000 pg/mL.



Supplemental Figure I Per-protocol consort diagram

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): screening and randomization. The per-protocol analysis was performed on all compliant, validly randomized patients with minimum baseline and follow-up cardiovascular magnetic resonance (CMR).

Supplemental Table I

Eligibility criteria

Inclusion criteria

Women aged 18-70 years

Eastern Cooperative Oncology Group (ECOG) performance status 0–1

Serum creatinine < 1.6 mg/dL or estimated glomerular filtration rate (eGFR) \geq 60 ml/min/1.73 m²

Systolic blood pressure \geq 110 mm Hg and < 170 mm Hg

Left ventricular ejection fraction \geq 50%

Exclusion criteria

Hypotension, defined as systolic blood pressure < 110 mmHg

Prior anthracycline chemotherapy regimen

Prior malignancy requiring chemotherapy or radiotherapy

Symptomatic heart failure

Systolic dysfunction (left ventricular ejection fraction < 50%)

Clinically significant coronary artery disease, valvular heart disease, significant arrhythmias, or conduction delays

Bradycardia, defined as heart rate < 50 b.p.m.

Uncontrolled arterial hypertension defined as systolic blood pressure > 170 mm Hg

Treatment with angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or beta-blocker within the last 4 weeks prior to study start

Intolerance to angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or beta-blocker

Uncontrolled concomitant serious illness, as determined by the investigator

Pregnancy or breastfeeding

Active abuse of drugs or alcohol

Suspected poor compliance

Inability to tolerate the MRI protocol

Supplemental Table II Tumor characteristics

	Candesartan- Metoprolol	Candesartan- Placebo	Placebo- Metoprolol	Placebo- Placebo
N	28	32	30	30
Mastectomy	8 (28.6%)	8 (25.0%)	14 (46.7%)	15 (50.0%)
Right Side	16 (57.1%)	16 (50.0%)	18 (60.0%)	16 (53.3%)
Left side	11 (39.3%)	16 (50.0%)	10 (33.3%)	145 (46.7%)
Bilateral	1 (3.6%)	0 (0%)	2 (6.7%)	0 (0%)
Tumor size:				
Tumor not found	0 (0.0%)	2 (6.3%)	0 (0%)	0 (0%)
T1	14 (50.0%)	17 (53.1%)	15 (50.0%)	12 (40.0%)
T2	14 (50.0%)	12 (37.5%)	15 (50.0%)	17 (56.7%)
T3	0 (0%)	1 (3.1%)	0 (0%)	1 (3.3%)
Tumor grade				
G0	0 (0%)	1 (3.1%)	0 (0%)	0 (0%)
G1	0 (0%)	2 (6.3%)	0 (0%)	1 (3.3%)
G2	12 (42.9%)	11 (34.4%)	14 (46.7%)	9 (30.0%)
G3	16 (57.1%)	18 (56.3%)	16 (53.3%)	20 (66.7%)
Lymph node				
N0	16 (57.1%)	20 (62.5%)	17 (56.7%)	17 (56.7%)
N1	10 (35.7%)	7 (21.9%)	7 (23.3%)	9 (30.0%)
N2	2 (7.1%)	5 (15.6%)	5 (16.7%)	2 (6.7%)
N3	0 (0%)	0 (0%)	1 (3.3%)	2 (6.7%)
Immunohistology				
HER 2 positive*	7 (25.0%)	7 (21.9%)	6 (20.0%)	8 (26.7%)
Estrogen Receptor negative (< 1%)	7 (25.0%)	8 (25.0%)	6 (20.0%)	10 (33.3%)
Estrogen Receptor positive >1% and <50%	1 (3.6%)	0 (0%)	3 (10.0%)	2 (6.7%)
Estrogen Receptor positive ≥50%	20 (71.4%)	24 (75%)	21 (70.0%)	18 (60.0%)
Progesterone Receptor positive (>10%)	16 (57.1%)	20 (62.5%)	16 (53.3%)	19 (63.3%)
Ki 67 ≥ 30 %	16 (57.1%)	22 (68.8%)	17 (56.7%)	17 (56.7%)
Ki 67 < 30%	3 (10.7%)	7 (21.9%)	9 (30.0%)	7 (23.3%)
Not measured	9 (32.1%)	3 (9.4%)	4 (13.3%)	6 (20.0%)
Treatment				
FEC† 240 mg/m ² (161 mg/m ² DE‡)	17 (60.7%)	19 (59.4%)	17 (56.7%)	15 (50.0%)
FEC 360 mg/m ² (241 mg/m ² DE)	4 (14.3%)	6 (18.8%)	6 (16.7%)	6 (20.0%)
FEC 400 mg/m ² (268 mg/m ² DE)	7 (25.0%)	7 (21.9%)	6 (20.0%)	7 (23.3%)
Incomplete FEC treatment	0 (0%)	0 (0%)	2 (6.7%)	2 (6.7%)
Trastuzumab	7 (25.0%)	7 (21.9%)	6 (20.0%)	7 (23.3%)
Incomplete Trastuzumab	0 (0%)	0 (0%)	1 (3.3%)	0 (0%)
Taxol	21 (75.0%)	22 (68.8%)	21 (70.0%)	15 (50.0%)
Taxotere	1 (3.6%)	1 (3.1%)	2 (6.7%)	4 (13.3%)
Incomplete Taxane treatment	2 (7.1%)	2 (6.3%)	2 (6.7%)	3 (10.0%)
Radiation	16 (67.1%)	19 (59.4%)	20 (66.7%)	21 (70.0%)
Left side	4/16 (25.0%)	11/19 (57.9%)	6/20 (30.0%)	7/21 (33.3%)
Right side	12/16 (75.0%)	8/19 (42.1%)	14/20 (70.0%)	14/21 (66.7%)

* HER 2 positive denotes immunohistochemistry (IHC) 3+ or IHC 2+ and amplified (ISH)

† FEC: fluorouracil, epirubicin, cyclophosphamide. Accumulated epirubicin dose. There were no significant differences between the four study groups

‡ DE: Doxorubicin equivalents