Supplemental Online Content

Vollenbroich R, Deckarm S, Gräni C, et al. Effect of paroxetine-mediated G-protein receptor kinase 2 inhibition vs placebo in patients with anterior myocardial infarction: a randomized clinical trial. *JAMA Cardiol*. Published online July 14, 2021. doi:10.1001/jamacardio.2021.2247

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

eMethods

Inclusion and Exclusion Criteria

Patients ≥18 years of age presenting with acute anterior ST-segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention within 24 hours of symptom onset were eligible if they had a left ventricular ejection fraction of ≤45% as assessed between 48 and 96 hours after revascularization. Subjects with previous myocardial infarction or previous revascularization procedures, known intolerance to paroxetine, contraindications to selective serotonin reuptake inhibitors, medical treatment with monoamine oxidase inhibitors within 14 days, contraindications to cardiac magnetic resonance imaging (CMR), renal failure, hepatic dysfunction, inability to follow study procedures, and women who were pregnant or breast feeding were excluded.

Safety population

The safety population included all study participants that took at least one dosage of paroxetine in the experimental arm.

Investigational Product and Study Procedures

In order to achieve serum paroxetine levels comparable to the ones documented in animal studies, we chose a paroxetine dosage of 20 mg daily, which is consistent with the recommended dose of paroxetine for the treatment of depression.[1] A taper dose of 10 mg daily was used for the duration of 7 days after completion of the treatment regimen in order to avoid withdrawal symptoms after discontinuation.

All study participants were asked to return the empty medication bottles (with remaining pills). Non-compliance was documented in case of discontinuation or interruption of the study drug, or a serum paroxetine level <6 ng/ml in patients in the experimental arm.

Cardiac Magnetic Resonance Imaging (CMR) Acquisition and Analysis

Images were obtained with a 1.5-T system (Magnetom Aera, Siemens Healthineers, Erlangen, Germany). All examinations included ciné images for the assessment of ventricular function. For the late gadolinium enhancement (LGE) images, intravenous of 0.2 mmol/kg of gadobutrol (Gadovist; Bayer Schering Pharma, Berlin-Wedding, Germany) was administered. LGE images were then acquired 10-15minutes post gadolinium injection. Images for both sequences were acquired in a short-axis stack with 8mm slice thickness to coverage of the entire ventricle without gap, and in 3 long-axis views.

A dedicated post-processing software (Circle, cvi42, Calgary, Canada, build 5.12) was used for image analysis. Ventricular volumes and morphology were quantified from the cine images. Semi-automated LGE-quantification was performed as follows: Epicardial and endocardial LV contours are carefully placed manually on all LGE images.[2] The remote normal, reference region was placed in a non-infarcted area without enhancement. LGE mass was then quantified as recommended by the society of cardiovascular magnetic resonance imaging by semi-automatic methods using a signal intensity threshold of 5-SD, respectively above a reference region of remote non-infarcted myocardium and areas of microvascular obstruction were included.[3] LGE is reported in grams and percentage relative to the entire LV mass.

eReferences

1. Schumacher SM, Gao E, Zhu W, et al. Paroxetine-mediated GRK2 inhibition reverses cardiac dysfunction and remodeling after myocardial infarction. Sci Transl Med. 2015;7(277):277ra31. doi:10.1126/scitranslmed.aaa0154

2. Gräni C, Eichhorn C, Bière L, et al. Comparison of myocardial fibrosis quantification methods by cardiovascular magnetic resonance imaging for risk stratification of patients with suspected myocarditis. J Cardiovasc Magn Reson Off J Soc Cardiovasc Magn Reson. 2019;21(1):14. doi:10.1186/s12968-019-0520-0

3. Schulz-Menger J, Bluemke DA, Bremerich J, et al. Standardized image interpretation and post-processing in cardiovascular magnetic resonance - 2020 update: Society for Cardiovascular Magnetic Resonance (SCMR): Board of Trustees Task Force on Standardized Post-Processing. J Cardiovasc Magn Reson. 2020;22(1). doi:10.1186/s12968-020-00610-6

eTable 1. Baseline Characteristics

	Paroxetine (N = 25)	Placebo (N = 25)
Age, years	62.2 ± 12.9	61.4 ± 12.5
Female sex	5 (20%)	4 (16%)
Body mass index, kg/m^2	27.6 ± 4.4	26.7 ± 4.9
Medical history		
Family history of CAD	5 (20%)	6 (24%)
Peripheral arterial disease	1 (4%)	0 (0%)
Insulin-treated	1 (4%)	3 (12%)
Hypertension	11 (44%)	12 (48%)
Hypercholesterolemia	11 (44%)	9 (36%)
Diabetes mellitus	2 (8%)	6 (24%)
Active smoker	6 (24%)	7 (28%)
History of smoking	2 (8%)	5 (20%)
Previous MI	0 (0%)	0 (0%)
Previous PCI or CABG	0 (0%)	0 (0%)
eGFR <60 ml/min	0 (0%)	4 (16%)
Renal insufficiency requiring dialysis	0 (0%)	0 (0%)
History of malignancy	3 (12%)	2 (8%)
Chronic obstructive lung disease	0 (0%)	0 (0%)
History of stroke or TIA	0 (0%)	2 (8%)
History of atrial fibrillation/atrial flutter	1 (4%)	0 (0%)
History of systemic inflammatory disease	1 (4%)	0 (0%)
Pacemaker	0 (0%)	0 (0%)

Data are presented stratified by allocated study drug according to the intention to treat principle. Data are expressed as n (%) or means±standard deviations. CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; TIA, transient ischemic attack.

eTable 2. Procedural Characteristics

	Paroxetine (n=25)	Placebo (n=25)
Killip class III or IV	5 (20%)	3 (12%)
Door-to-balloon time, min	49 ± 27	49 ± 27
Symptom onset-to-balloon time, min	573 ± 624	338 ± 381
LVEF by ventriculography, %	34.8 ± 7.3	32.7 ± 7.8
Total contrast, mL	194.6 ± 50.6	205.76 ± 66.4
Hemodynamic assist device	0 (0%)	1 (4%)
Culprit vessel		
Left main	0 (0%)	1 (4%)
Left anterior descending artery	25 (100%)	24 (96%)
TIMI 3 flow at the end of procedure	25 (100%)	25 (100%)
Vessels with significant lesions		
Left main	2 (8%)	1 (4%)
Left anterior descending artery	25 (100%)	24 (96%)
Ramus intermedius	1 (4%)	2 (8%)
Left circumflex	4 (16%)	6 (24%)
Right coronary artery	10 (40%)	10 (40%)
Vessels with PCI		
Left main	2 (8%)	1 (4%)
Left anterior descending artery	25 (100%)	24 (96%)
Ramus intermedius	0 (0%)	2 (8%)
Left circumflex	1 (4%)	1 (4%)
Right coronary artery	0 (0%)	1 (4%)
Procedural medications (within 24h)		
Aspirin	25 (100%)	25 (100%)
Loading with clopidogrel	2 (8%)	1 (4%)
Loading with ticagrelor	17 (68%)	20 (80%)
Loading with prasugrel	6 (24%)	4 (16%)
Unfractionated heparin	25 (100%)	25 (100%)
GP IIb/IIIa	7 (28%)	15 (60%)

Data are presented stratified by allocated study drug according to the intention to treat principle. Data are expressed as n (%) or means±standard deviations. CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; TIA, transient ischemic attack. Data expressed as n (%) or means±standard deviations. IABP: intra-aortic balloon pump; ECMO: extracorporeal membrane oxygenation; PTCA: percutaneous transluminal coronary angioplasty.

eTable 3. Medication

	At discharge		At 12 weeks	
	Paroxetine (n=25)	**Placebo (n=25)**	**Paroxetine (n=23)**	**Placebo (n=23)**
Aspirin	25 (100%)	25 (100%)	21 (91%)	21 (91%)
Clopidogrel	5 (20%)	6 (24%)	8 (35%)	7 (30%)
Prasugrel	6 (24%)	4 (16%)	4 (17%)	5 (22%)
Ticagrelor	14 (56%)	15 (60%)	9 (39%)	11 (48%)
Vitamin K Antagonist	1 (4%)	0 (0%)	1 (4%)	0 (0%)
NOAC	4 (16%)	6 (24%)	7 (30%)	5 (22%)
Statin	24 (96%)	25 (100%)	22 (96%)	22 (96%)
Other lipid lowering drug	1 (4%)	2 (8%)	0 (0%)	2 (9%)
ACE inhibitor	18 (72%)	18 (72%)	14 (61%)	17 (74%)
AT II antagonist	7 (28%)	7 (28%)	8 (35%)	6 (26%)
Beta blocker	25 (100%)	25 (100%)	21 (91%)	23 (100%)
Aldosteron antagonist	10 (40%)	11 (44%)	9 (39%)	9 (39%)
Sacubitril/Valsartan	3 (12%)	3 (12%)	4 (17%)	2 (9%)
SGLT-2 inhibitor	3 (12%)	4 (16%)	3 (13%)	1 (4%)
Calcium-antagonist	1 (4%)	0 (0%)	1 (4%)	2 (9%)
Amiodarone	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Digoxin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nitrates	1 (4%)	0 (0%)	0 (0%)	0 (0%)
Diuretics	14 (56%)	14 (56%)	10 (43%)	9 (39%)
Insulin	0 (0%)	3 (12%)	1 (4%)	3 (13%)
Proton-pump inhibitor	20 (80%)	21 (84%)	16 (70%)	11 (48%)

Data are presented stratified by allocated study drug according to the intention to treat principle. Data are expressed as n (%). NOAC, non-vitamin K oral anticoagulant; ACE, angiotensin-converting enzyme; AT, angiotensin; SGLT, sodium dependent glucose transporter.

eTable 4. Baseline characteristics in patients with versus without CMR at follow-up

	All patients N = 50	With CMR N = 38	Without CMR N = 12	p-value
Age, years	61.8 ± 12.6	60.6 ± 10.8	65.8 ± 17.0	0.215
Female sex	9 (18%)	8 (21%)	1 (8%)	0.425
Medical history				
Hypertension	23 (46%)	16 (42%)	7 (58%)	0.508
Hypercholesterolemia	20 (40%)	14 (37%)	6 (50%)	0.506
Diabetes mellitus	9 (18%)	6 (16%)	3 (25%)	0.668
GFR < 60ml/min	4 (8%)	2 (5%)	2 (17%)	0.240
History of afib/aflut	1 (2%)	1 (3%)	0 (0%)	1.000
Characteristics of myocardial infarction				
Killip class III or IV	8 (16%)	5 (13%)	3 (25%)	0.379
Culprit vessel				
Left main	1 (2%)	1 (3%)	0 (0%)	1.000
Left anterior descending artery	49 (98%)	37 (97%)	12 (100%)	1.000
Multivessel coronary artery disease	24 (48%)	17 (45%)	7 (58%)	0.514
CK peakl, U/L	3573 ± 2394	3223 ± 2291	4679 ± 2474	0.066

Heart failure medication at discharge				
ACE inhibitor or AT II antagonist	50 (100%)	38 (100%)	12 (100%)	.
Beta blocker	50 (100%)	38 (100%)	12 (100%)	.
Aldosterone antagonist	21 (42%)	13 (34%)	8 (67%)	0.091
Sacubitril/Valsartan	6 (12%)	4 (11%)	2 (17%)	0.621
SGLT-2 inhibitor	7 (14%)	5 (13%)	2 (17%)	1.000

Data expressed as n (%, p-value from Fisher's tests) or means±standard deviations (p-value from unpaired t-tests).

eTable 5. Echocardiography

	Paroxetine				Placebo				Paroxetine vs Placebo	
	Baseline	Week 12	Change	p-value	Baseline	Week 12	Change	p-value	Mean difference of the change (95% CI)	p-value
Ventricular function										
LV-ejection fraction (Simpson), %	n=22, 41.9 ±9.0	n=19, 48.6 ±11.4	n=18, 5.9 ±10.9	0.034	n=21, 42.2 ±8.8	n=18, 51.9 ±10.8	n=14, 8.6 ±10.5	0.009	n=32, -2.7 (-10.5 to 5.1)	0.48
LV-enddiastolic diameter, mm	n=21, 49.8 ±6.0	n=17, 52.2 ±6.1	n=15, 2.6 ±6.3	0.13	n=20, 49.4 ±5.6	n=18, 50 ±7.7	n=14, -0.2 ±5.3	0.88	n=29, 2.8 (-1.61 to 7.2)	0.21
Valvular heart disease										
AS, moderate or severe	n=25, 1 (4%)	n=22, 0 (0%)			n=23, 0 (0%)	n=20, 0 (0%)				0.47*
AR, moderate or severe	N=25, 0 (0%)	n=22, 0 (0%)			n=23, 0 (0%)	n=20, 0 (0%)				0.47*
MR, moderate or severe	n=25, 1 (2.1%)	n=22, 4 (9.5%)			n=23, 0 (0%)	n=20, 2 (9.1%)				0.21*
MS, moderate or severe	n=25, 0 (0%)	n=22, 0 (0%)			n=23, 0 (0%)	n=20, 0 (0%)				0.22*
TR, moderate or severe	n=25, 1 (4%)	n=22, 1 (4.5%)			n=23, 1 (4.3%)	n=20, 2 (10%)				0.59*

Data are presented stratified by allocated study drug according to the intention to treat principle. Data are expressed as means±standard deviations or n (%). 95% CI, 95% confidence interval; LV, left-ventricular; AS, aortic stenosis; AR, aortic regurgitation; MR, mitral regurgitation; MS, mitral stenosis; TR, tricuspid regurgitation; na, not applicable. * comparing moderate or severe versus none or mild between the two groups.

eTable 6. Clinical Events

	Paroxetine (n=23)	Placebo (n=23)
Transient ischemic attack[a]	1 (4%)*	0 (0%)
Acute renal failure[b]	1 (4%)*	0 (0%)
Congestive heart failure requiring rehospitalisation[c]	0 (0%)	1 (4%)
Infarct-associated pericarditis[d]	0 (0%)	1 (4%)*
Pneumonia[e]	0 (0%)	1 (4%)*
Other events of interest		
LV-aneurysm requiring surgical aneurysmectomy	1 (4%)*	0 (0%)
Electrical storm requiring ablation and ICD implantation	1 (4%)*	0 (0%)
EP study with inducible monomorphic tachycardia followed by ICD implantation	0 (0%)	1 (4%)*
Coronary artery bypass grafting	0 (0%)	1 (4%)

Data are presented stratified by allocated study drug according to the intention to treat principle and expressed as n (%). LV, left-ventricular; ICD, implantable cardioverter defibrillator; EP, elecrtrophysiology. * event occurred in the safety population. [a] defined as a brief episode of neurological dysfunction resulting from focal cerebral ischemia not associated with permanent cerebral infarction. [b] "Injury" according to RIFLE criteria. [c] defined as hospitalization due to clinical symptoms of congestive heart failure with clinical signs including pulmonary edema, hypoperfusion or documented volume overload AND administration of IV diuresis or inotropic therapy. [d] chest pain and pericardial effusion three weeks after STEMI; no new findings on coronary angiogram, symptoms resolved with anti-inflammatory treatment. [e] patient treated at external hospital with antibiotics in the setting of elevated CRP and pulmonary infiltrates.

eTable 7. Reported Side Effects

	Paroxetine (n=23)	Placebo (n=23)
Loss of appetite	1 (4%)	0 (0%)
Fatigue	1 (4%)	0 (0%)
Tremor	1 (4%)	0 (0%)
Urinary urgency	1 (4%)	0 (0%)
Dry eyes	1 (4%)	0 (0%)
Increased sweating	0 (0%)	1 (4%)
Erectile dysfunction	0 (0%)	1 (4%)

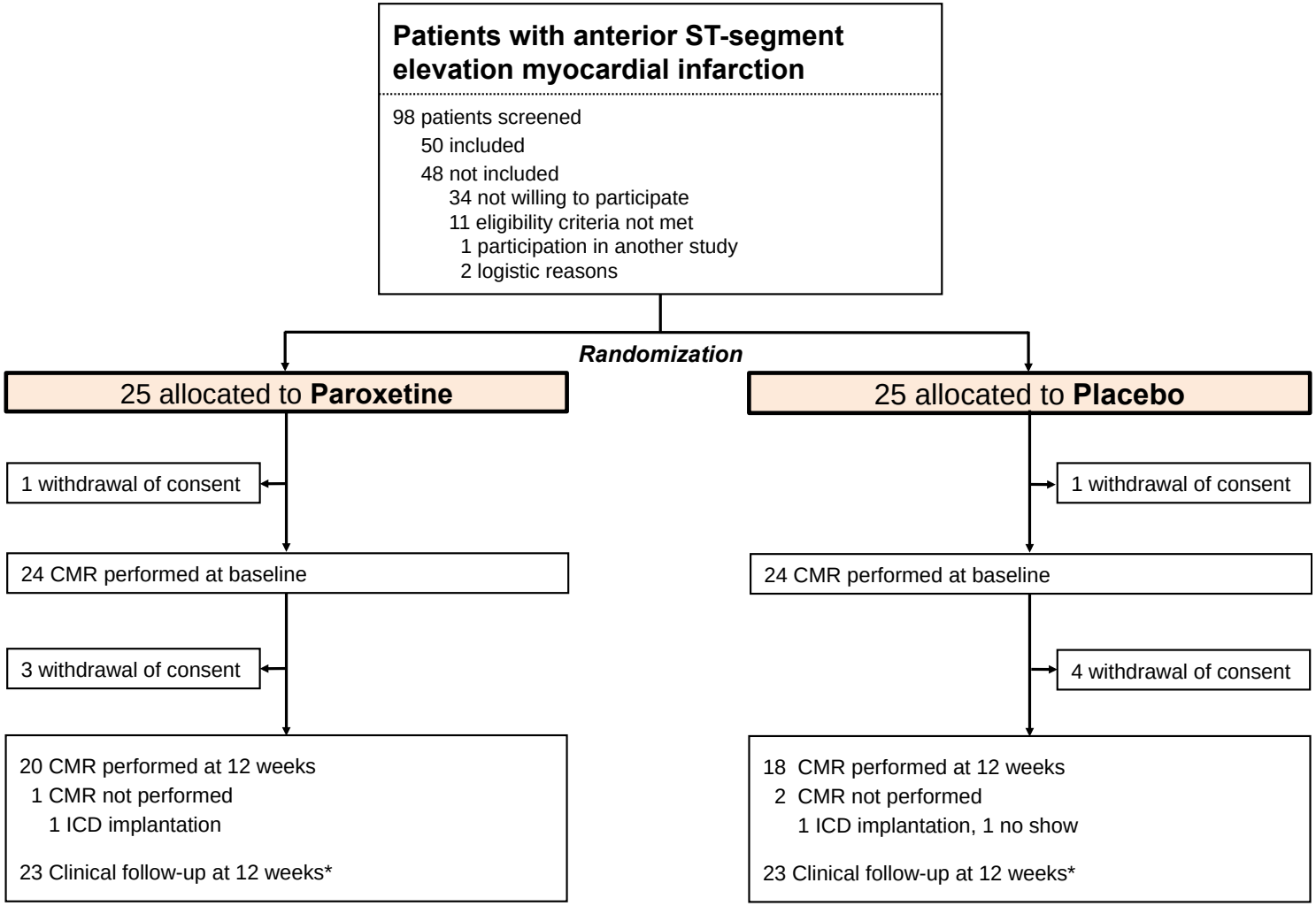
Data are presented stratified by allocated study drug according to the intention to treat principle and expressed as n (%).

eTable 8. CMR Results in Per-Protocol Population.

	Paroxetine				Placebo				Paroxetine vs Placebo	
	Baseline n=20	Week 12 n=19	Change n=19	p-value	Baseline n=17	Week 12 n 16	Change n=16	p-value	Mean difference of the change (95% CI) n=35	p-value
LV dimensions and function										
EDD, mm	51.5 ±6.5	53.1 ±8.9	1.5 ±6.1	0.29	48.5 ±8.2	51.6 ±6.9	2.9 ±7.5	0.14	-1.4 (-6.0 to 3.3)	0.70
ESD, mm	35.0 ±7.3	37.3 ±9.5	1.8 ±7.5	0.32	33.7±8.8	33.8 ±8.9	-0.3 ±7.6	0.88	2.0 (-3.2 to 7.3)	0.43
EDV, mL	189.5 ±42.3	204.8 ±62.8	14.9 ±45.6	0.17	175.2 ±34.1	181.2 ±51.6	7.9 ±29.0	0.29	7.0 (-19.8 to 33.9)	0.60
ESV, mL	112.1 ±34.4	111.7 ±43.0	-0.35 ±21.1	0.94	103.3 ±30.4	95.7 ±36.5	-5.5 ±16.5	0.20	5.2 (-8.1 to 18.4)	0.43
SV, mL	77.4 ±21.4	93.2 ±28.0	15.3 ±28.3	0.03	72.0±16.9	85.2 ±23.0	13.1 ±17.2	0.008	2.2 (-14.3 to 18.7)	0.79
Mass, g	136.9 ±35.7	121.7 ±35.2	-14.3 ±26.3	0.029	126.2±23.8	111.7 ±21.2	-13.7 ±13.0	0.001	-0.6 (-15.3 to 14.1)	0.93
CO, L/min	5.3 ±1.2	5.5 ±1.9	0.2 ±1.9	0.62	5.3 ±1.1	5.5 ±1.4	0.3 ±1.0	0.27	-0.1 (-1.1 to 0.99)	0.91
EF, %	41.4 ±8.5	46.4 ±8.5	4.9 ±5.9	0.002	41.8 ±9.3	48.1 ±9.7	5.8 ±5.2	<0.001	-0.9 (-4.8 to 2.9)	0.63
Late gadolinum enhancement										
5SD, total g	50.8 ±23.1	31.2 ±16.1	-20.1 ±16.7	<0.001	42.4 ±19.4	30.0 ±16.0	-9.7 ±12.2	0.006	-10.4 (-20.6 to -0.2)	0.047
5SD, % of total g	38.4 ±11.7	24.6 ±9.9	-14.3 ±12.9	<0.001	34.8±12.4	28.9 ±13.6	-4.8 ±9.3	0.06	-9.4 (-17.3 to -1.6)	0.021

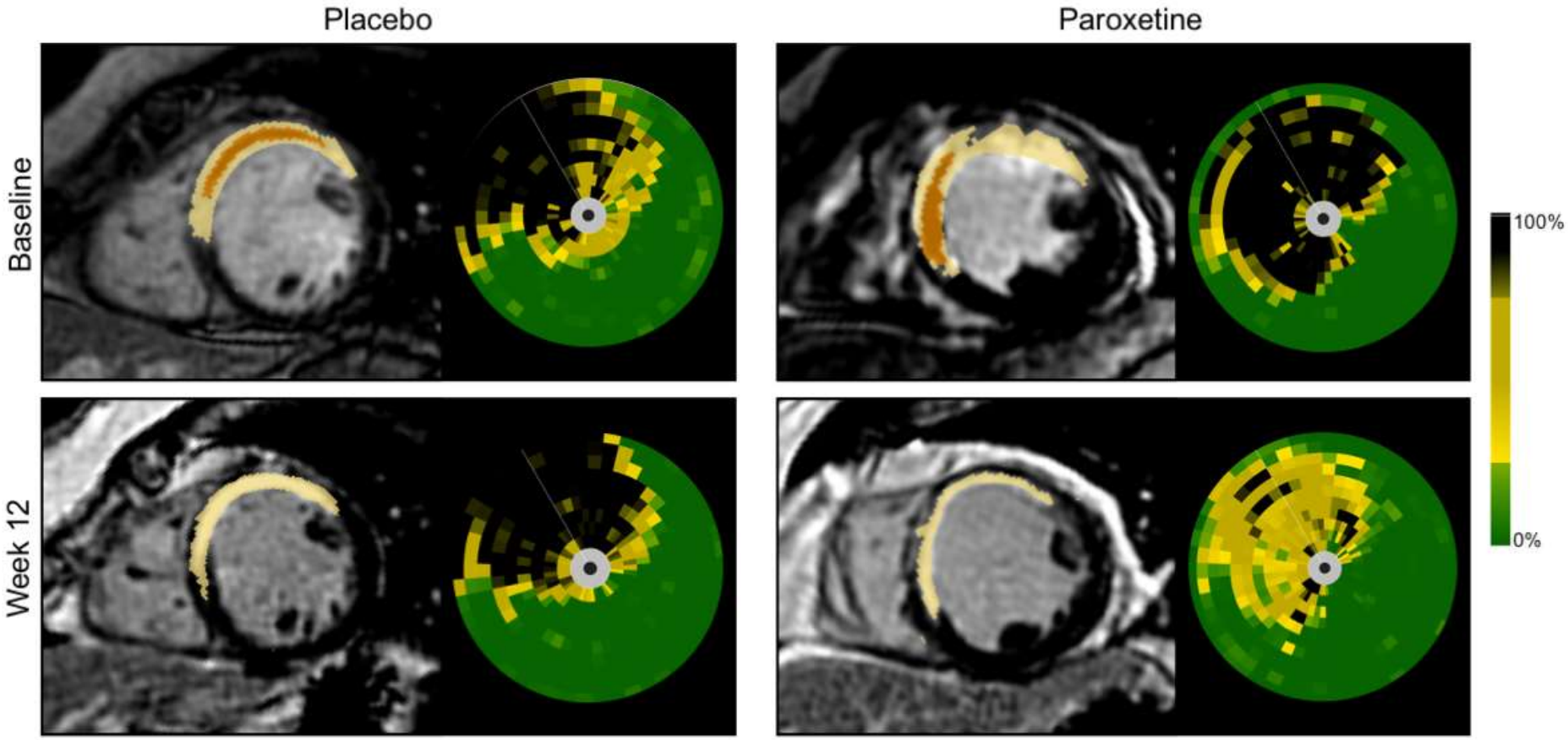
Data are presented stratified by study medication according to the per-protocol principle comprising all subjects that underwent baseline and follow-up cardiac magnetic resonance imaging (CMR), had measurable paroxetine levels or returned less than 20% of the study medication. Data are expressed as means±standard deviations (SD) 95% CI, 95% confidence interval; LV, left-ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CO, cardiac output; EF, ejection fraction.

eFigure 1. Flowchart according to CONSORT statement.



The flowchart illustrates screening, treatment allocation, withdrawals and outcome assessment. CMR, cardiac magnetic resonance imaging; ICD, implantable cardioverter defibrillator.

eFigure 2. Examples of LGE in CMR



Two examples of magnetic resonance imaging (CMR) with late gadolinium enhancement (LGE) in the paroxetine and placebo group. Presented are shortaxis LGE slices showing region of enhancement 5SD above the reference range (yellow), with orange depicting territories with microvascular obstruction. Bullseye plots demonstrate relative enhancement in percent of myocardial mass from the base (outer ring) to the apex (inner ring). In the presented patient with paroxetin, LGE decreased from 48% (69g) of total left ventricular mass at baseline to 23% (37g) at 12 week follow-up, in the patient taking placebo the corresponding decrease of LGE was 46% (53g) to 40% (35g).