Clinical Study Protocol

Paroxetine-mediated GRK2 inhibition to reduce cardiac remodeling after acute myocardial infarction (CARE-AMI): a randomized controlled pilot study

Study Type:	*Clinical trial with Paroxetine (IMP) in Acute ST-Segment Elevation Myocardial Infarction*
Study Categorisation:	*Risk category B*
Study Registration:	*NCT03274752*
Study Identifier:	*None*
Sponsor-Investigator:	Prof. Dr. med. Thomas Pilgrim Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern (Switzerland) Phone: 41 31 632 08 27 Fax: 41 31 632 47 70 e-mail: thomas.pilgrim@insel.ch
Investigational Product:	*Paroxetine (Deroxat)*
Protocol Version and Date:	*Version 7.0, 24.01.2020*

CONFIDENTIAL

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Study number	*The study will be registered with ClinicalTrials.gov.*
Study Title	Paroxetine-mediated GRK2 inhibition to reduce cardiac remodeling after acute myocardial infarction (CARE-AMI): a randomized controlled pilot study

The Sponsor-Investigator and trial statistician have approved the protocol version 7 (dated 24.01.2020)], and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor-Investigator: Prof. Dr. med. Thomas Pilgrim

Place/Date	Signature

Local Principal Investigator at study site*:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	Bern University Hospital Freiburgstrasse 10 3010 Bern, Switzerland
Principal investigator	*Prof. Dr. med. Thomas Pilgrim*

Place/Date	Signature

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	*Prof. Dr. med. Thomas Pilgrim*
Study Title:	Paroxetine-mediated GRK2 inhibition to reduce cardiac remodeling after acute myocardial infarction (CARE-AMI): a randomized controlled pilot study
Short Title / Study ID:	Cardiac Remodeling after Acute Myocardial Infarction (CARE-AMI)
Protocol Version and Date:	*Version 7.0, 24.01.2020*
Trial registration:	*NCT03274752*
Study category	*Risk category B*
Clinical Phase:	*Phase 3 study*
Background and Rationale:	Cardiac remodeling is characterized by a composite of structural, geometric, molecular, and functional changes of the myocardium, and is an important determinant of heart failure and cardiovascular outcome in survivors of acute myocardial infarction. Progression of heart failure secondary to the remodeling process results from dysregulation of the G protein-coupled receptor (GPCR). Excessive adrenergic drive in patients with heart failure results in an enhanced activation of GPCR kinases (GRKs) that is considered to have a central role in adverse cardiac remodeling after ischemic injury. The selective Serotonin reuptake inhibitor paroxetine specifically binds to the catalytic domain of GRK2 as an off-target effect, and has been shown to reverse cardiac remodeling and increase left ventricular ejection fraction in a mouse model. The effect was observed at serum levels achieved with standard dosages of paroxetine, and was robust in mice with and without concomitant heart failure treatment, respectively.
Objective(s):	The objective of the present study is to investigate the off-target effect of paroxetine to reverse cardiac remodeling and improve left ventricular ejection fraction in patients after acute myocardial infarction.

Endpoints:	**Primary Endpoint:** - difference in the change (Δ) in LVEF at 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI (CMR). **Secondary Endpoints:** - in left left-ventricular end-diastolic volume (LVEDV) between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI. - in left left-ventricular end-systolic volume (LVESV) between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI. - in late-enhancement between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI. - in LVEF between baseline and 12 weeks, and 12 months, respectively in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by transthoracic echocardiography. - major adverse cardiac events (cardiac death, myocardial infarction, repeat hospitalization for heart failure) at 12 weeks and 12 months, respectively. - clinical symptoms of heart failure as assessed by New York Heart Association (NYHA) at 12 weeks and 12 months, respectively.
Study design:	*Prospective, randomized, placebo controlled, double-blinded, explorative single-center study*
Inclusion / Exclusion criteria:	*Inclusion criteria:* 1. Age ≥18 years 2. Anterior wall ST-segment elevation myocardial infarction 3. Primary PCI within 24 hours of symptom onset 4. LVEF ≤ 45% within 48-96 hours after primary PCI (TTE) *Exclusion criteria:* 1. Female patients at reproductive age (<50 years) 2. Known intolerance to paroxetine 3. Inability to provide informed consent 4. Currently participating in another trial before reaching first endpoint 5. Current medical therapy with MAO-blocker (during, 14 days before, and 14 days after treatment with MAO-blocker), lithium, thioridazide, or pimozide 6. Concomitant tamoxifen intake 7. Previous myocardial infarction 8. Previous revascularization procedure (percutaneous coronary intervention or coronary artery bypass grafting). 9. Contraindication to CMR 10. Obvious or questionable inability to appropriately cooperate (alcohol, drugs etc.) 11. Relevant nephropathy or hepatopathy

Measurements and procedures:	LVEF after myocardial infarction will be measured within a window of 2-6 days after primary PCI by the use of TTE and CMR. If LVEF $\leq$ 45%, patients will be randomized in a 1:1 ratio to either the treatment arm (paroxetine) or the control arm (Placebo). Study medications will be administered for the duration of twelve weeks, before LVEF will be re-assessed by CMR and TTE. Clinical and echocardiographic follow-up will be performed at 12 months after randomisation. In addition, paroxetine serum levels will be measured at 12 weeks. To avoid abrupt discontinuation of the study drug, the study dosage will be decreased by 50% after 12 weeks for a time period of one week, afterwards it will be completely stopped.
Study Product / Intervention:	*Paroxetine (Deroxat) will be administered in a dosage of 20mg q.d. per os continuously for 12 weeks after primary PCI. In week 13, Paroxetine (Deroxat) will be administered in a dosage of 10mg q.d. per os.*
Control Intervention:	*Placebo will be given q.d. per os continuously for 12 weeks after primary PCI. In addition, a placebo will be given q.d. per os in week 13 as well.*
Number of Participants with Rationale:	*For each arm of the study the estimated number of participants will be 25. We assume a difference in delta LVEF of 10% in the treatment group compared to the control group with a standard deviation of 10% (increase of 5% in the control group and 15% in the treatment group) after 12 weeks. Dropout rate at 12 weeks was estimated to be 10% in both groups. We calculated a sample size of 50 patients to provide more than 90% power to detect superiority on the primary endpoint at a two-sided type I error of 0.05.*
Study Duration:	*The study of an enrolment period (see Study Schedule) and a clinical follow-up duration of 12 months*
Study Schedule:	*First-Participant-In (planned): September 2017* *Last-Participant-Out (planned): December 2022*

Investigator(s):	*Principal Investigator:* Prof. Dr. med. Thomas Pilgrim Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern Switzerland Phone: 41 31 632 08 27 Fax: 41 31 632 47 70 e-mail: thomas.pilgrim@insel.ch *Co-Investigator:* Dr. med. René Vollenbroich, MPP Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern Switzerland Phone: 41 31 632 21 11 Fax: 41 31 632 47 70 e-mail: rene.vollenbroich@insel.ch *Co-Investigator:* PD Dr. med. Lukas Hunziker-Munsch Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern Switzerland Phone: 41 31 632 82 84 Fax: 41 31 632 47 70 e-mail: lukas.hunziker@insel.ch *Co-Investigator:* Prof. Dr. med. Stephan Windecker Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern Switzerland Phone: 41 31 632 9653 Fax: 41 31 632 47 70 e-mail: stephan.windecker@insel.ch *Co-Investigator:* PD Dr. med. Marco Valgimigli Department of Cardiology Bern University Hospital Freiburgstrasse 10 3010 Bern Switzerland Phone: 41 31 632 3040 Fax: 41 31 632 47 70 e-mail: marco.valgimigli@insel.ch
Study Centre(s):	*Single-center (Bern University Hospital)*
Statistical Considerations:	All patients who undergo randomisation will be included in both the primary analysis of LVEF assessment as well as in the secondary analyses.

GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

STUDY SUMMARY IN LOCAL LANGUAGE

Hintergrund

Kardiales Remodeling ist durch strukturelle, geometrische, molekulare und funktionelle Veränderungen des Myokards charakterisiert und stellt einen wichtigen Faktor für die Diagnose einer Herzinsuffizienz sowie für die kardiovaskulären Folgen bei Patienten dar, welche einen akuten Myokardinfarkt überlebt haben. Die Entwicklung einer Herzinsuffizienz durch Remodelingprozesse beruht auf einer Dysregulation des G-Protein-gekoppelten Rezeptors (GPCR). Bei herzinsuffizienten Patienten sorgt die exzessive adrenerge Stimulation für eine vermehrte Aktivierung von GCPR Kinasen (GRKs), welche nach einem ischämischen Ereignis eine zentrale Rolle im Prozess des kardialen Remodelings einzunehmen scheinen. Paroxetin als selektiver Serotonin-Aufnahmehemmer bindet spezifisch an die katalytische Domäne von GRK2 im Sinne einer Off-Target-Aktivität. In einem Mausmodell konnte diesbezüglich ein reversibler Einfluss auf das kardiale Remodeling verbunden mit einer Verbesserung der linksventrikulären Ejektionsfraktion (LVEF) gezeigt werden. Dieser Effekt wurde unter Standarddosierung von Paroxetin mithilfe von Serumanalysen analysiert und blieb bei Mäusen mit und ohne begleitende Herzinsuffizienztherapien stabil.

Ziel

Das Ziel der vorliegenden Studie ist es, den Off-Target-Effekt von Paroxetin hinsichtlich der Reduktion des kardialen Remodelings und der Verbesserung der LVEF bei Patienten mit akutem Myokardinfarkt zu untersuchen.

Methoden

Die Paroxetine-mediated GRK2 inhibition to reduce cardiac remodeling after acute myocardial infarction (CARE-AMI) – Studie ist eine doppelblind randomisierte und Placebo kontrollierte Pilotstudie (Phase3, Risikokategorie B). Patienten mit einem akuten Myokardinfarkt (<24h seit Symptombeginn) und primärer perkutaner Koronarintervention werden verblindet in einem 1:1 Verhältnis entweder in die Paroxetin- oder die Placebogruppe <7 Tage nach primärer Koronarintervention randomisiert. Die Randomisierung wird mithilfe von versiegelten Umschlägen vorgenommen. Die Behandlung besteht zusätzlich zur Standardbehandlung bei Myokardinfarkt aus Paroxetin 20mg einmal täglich oder Placebo einmal täglich für insgesamt 12 Wochen. Die LVEF wird am Anfang sowie nach 12 Wochen mittels transthorakaler Echokardiographie sowie kardialer Magnetresonanztomographie (cMRI) erhoben. Paroxetin Serumlevel werden ebenfalls nach 12 Wochen bestimmt. Ein telefonischer Follow-up erfolgt nach 4 Wochen, klinische Follow-ups erfolgen nach 12 Wochen und nach 12 Monaten. Der primäre Endpunkt ist der Unterschied zwischen der Differenz der LVEF bei Studieneinschluss und nach 12 Wochen erhoben durch cMRI. Sekundäre Endpunkte sind die Unterschiede des linksventrikulären enddiastolischen Volumens (LVEDV), des linksventrikulären endsystolischen Volumens (LVESV) und des Late-enhancements nach 12 Wochen im Unterhschied zu den Baseline-Werten. Diese Werte werden ebenfalls mittels cMRI erhoben. Zudem werden der Unterschied der LVEF (mithilfe transthorakaler Echokardiographie), schwerwiegende unerwünschte kardiovaskuläre Ereignisse (=major adverse cardiac events (MACE)), und die funktionelle New York Heart Association Gruppe nach 12 Wochen und 12 Monaten ermittelt. Wir nehmen eine Kohortengrösse von insgesamt 50 Patienten an, um einen absoluten LVEF-Anstieg von 10% bei einer Power von 90% aufzuzeigen. Die Einschlusskriterien beinhalten Alter ≥ 18 Jahre, anteriorer ST-Hebungsinfarkt, primäre perkutane Koronarintervention innerhalb der ersten 24h seit Symptombeginn sowie LVEF ≤ 45% innerhalb 48-96h nach primärer Koronarintervention. Als Ausschlusskriterien gelten Schwangerschaft oder stillende Patientinnen, bekannte Paroxetinunverträglichkeit, Unfähigkeit zu einer Einverständniserklärung, gleichzeitige parallele Studienteilnahme an einer anderen Studie vor Erreichen des ersten Endpunktes, gleichzeitige medikamentöse Therapie mit einem MAO-Hemmer, Lithium, Thioridazid, Pimozid oder Tamoxifen, zurückliegender Myokardinfarkt oder Revaskularisation und vorliegende Kontraindikationen für die Durchführung einer Magnetresonanztomographie.

Potentielle Bedeutung der Studie

Die Reduktion von kardialem Remodeling nach einem akuten Myokardinfarkt durch GRK2-Hemmung, einem Off-Target Effekt des Serotonin-Wiederaufnahmehemmers Paroxetin, könnte die Erholung der eingeschränkten linksventrikulären Ejektionsfraktion nach einem ischämischen Ereignis fördern und damit zu einer klinischen Verbesserung von Patienten mit akutem Myokardinfarkt führen.

ABBREVIATIONS

AE	Adverse Event
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CRF	Case Report Form
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin)*
CMRI	Cardiac magnetic resonance imaing
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
GCP	Good Clinical Practice
IB	Investigator's Brochure
Ho	Null hypothesis
H1	Alternative hypothesis
HFG	Humanforschungsgesetz (Law on human research)
HMG	Heilmittelgesetz
HRA	Federal Act on Research involving Human Beings
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention to treat
KlinV	Verordnung über klinische Versuche in der Humanforschung *(in English: ClinO, in French OClin)*
LPTh	Loi sur les produits thérapeutiques
LRH	Loi fédérale relative à la recherche sur l'être humain
LVEDV	Left ventricular enddiastolic volume
LVESV	Left ventricular endsystolic volume
LVEF	Left ventricular Ejection Fraction
MACE	Major adverse cardiac events
MD	Medical Device
MRI	Magnetic Resonance Imaging
OClin	Ordonnance sur les essais cliniques dans le cadre de la recherche sur l'être humain *(in German : KlinV, in English : ClinO)*
PI	Principal Investigator

SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TTE	Transthoracic Echocardiography

STUDY SCHEDULE

Study Periods	*Screening*	*Treatment, Intervention Period*				*Follow-up*
Visit	*1*	*2*		*3*	*4*	*5*
Time (hour, day, week)	*0 - 1*	*2-6d*	*<7d*	*4wks*	*12wks*	*12mnths*
Patient Information and Informed Consent	*X*					
Demographics	*X*					
Medical History	*X*					
In- /Exclusion Criteria	*X*					
Physical Examination	*X*				*X*	*X*
Vital Signs	*X*	*X*	*X*		*X*	*X*
*Laboratory Tests *)*	*X*				*X*	*X*
Serum Paroxetine Level					*X*	
ECG	*X*				*X*	*X*
Randomisation			*X*			
TTE		*X*			*X*	*X*
Cardiac MRI		*X*			*X*	
Administer Study Medication/Placebo			*X*			
Primary Variables	*X*	*X*			*X*	*X*
Secondary Variables	*X*				*X*	*X*
Follow Up Phone Call				*X*		
Adverse Events			*X*	*X*	*X*	*X*

*) CK, CK-MB, hs-Troponine T, NT-pro BNP, hemoglobine, hematocrit, leukocytes, thrombocytes, Glucose, HbA1c, creatinine, cholesterol, HDL, LDL, tryglycerides, ALAT, ASAT, AP, Gamma-GT, TSH

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator

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The sponsor-investigator is the principal investigator and has the final responsibility for the study design, data management, analysis, interpretation, and writing of the report.

1.2 Principal Investigator(s)

The principal investigator in this study equals the sponsor-investigator

1.3 Statistician ("Biostatistician")

Martina Rothenbühler, MSc
Cardiovascular Statistics
Clinical Trials Unit
University of Bern
Finkenhubelweg 11
CH-3012 Bern, Switzerland
Phone: 0041 31 631 44 85
martina.rothenbuehler@ctu.unibe.ch

1.4 Laboratory

Laboratory of the University Hospital of Bern
Freiburgstrasse 10
CH-3010 Bern Switzerland

1.5 Monitoring institution

Central data monitoring will be performed by the Clinical Trials Unit of the University of Bern.

1.6 Any other relevant Committee, Person, Organisation, Institution

Not applicable

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents shall be submitted to a properly constituted Competent Ethics Committee (CEC) and/or competent authorities (Swissmedic) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.

The decision of the CEC and Swissmedic/foreign competent authority concerning the conduct of the study is made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registered with ClinicalTrials.gov, as well as in the Swiss Federal Complementary Database.

2.2 Categorisation of study

Risk category B, since

- *Paroxetine is authorized in Switzerland*
- Indication is different from that specified in the prescribing information, and not within the same disease group

2.3 Competent Ethics Committee (CEC)

The responsible investigator ensures that approval from an appropriately constituted Competent Ethics Committee (CEC) is sought for the clinical study.

All changes in the research activity and all unanticipated problems involving risks to humans (including in case of planned or premature study end) and the final report will be reported/send to the CEC.

No changes are made to the protocol without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 2.10.

2.4 Competent Authorities (CA)

The Sponsor will obtain CA approval from the competent authority (Swissmedic) before the start of the clinical trial.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, in

case of medical device: the European Directive on medical devices 93/42/EEC and the ISO Norm 14155 and ISO 14971, the Swiss Law and Swiss regulatory authority's requirements. (1-7) The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

None of the authors of this study protocol indicates a conflict of interest.

2.7 Patient Information and Informed Consent

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

The participant must be informed that his/her medical records may be examined by authorised individuals other than their treating physician.

All participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. The time frame in which a participant can decides whether to participate or not will be up to 48 hours.

The patient information sheet and the consent form will be submitted to the CEC and to the competent authority (as applicable) to be reviewed and approved. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (e.g. Swissmedic), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of the clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

Protocol amendments

Substantial amendments are only implemented after approval of the CEC and CA respectively and may be done through the Sponsor-Investigator.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC/CA. Such deviations shall be documented and reported to the sponsor and the CEC/CA as soon as possible.

All Non-substantial amendments are communicated to the CA as soon as possible if applicable and to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

While cardiac mortality has substantially decreased over the past decades, cardiovascular disease continues to be the leading cause of mortality in the western world. Progress in timely myocardial reperfusion for acute myocardial infarction has importantly contributed to the reduction of cardiac mortality. Nevertheless, heart failure secondary to ischemic heart disease is an ongoing health care challenge, and alternative pathophysiological mechanisms are less well exploited in current therapeutic strategies. Survivors of acute myocardial infarction are at increased risk for the development of congestive heart failure secondary to a process referred to as cardiac remodeling.

Cardiac remodeling is characterized by a composite of structural, geometric, molecular, and functional changes of the myocardium, and is an important determinant of heart failure and cardiovascular outcome in survivors of acute myocardial infarction.(8) Dysregulation of G protein-coupled receptor (GPCR) is an important pathway of heart failure progression associated with the remodeling process. Overstimulation of beta-adrenergic receptors results in an enhanced activation of GPCR kinases (GRKs), which translates into a loss in inotropic reserve.(9) Failing human hearts have a two to three fold increase in GRK2 activity, which is considered to have a central role in adverse cardiac remodeling after ischemic injury.(10) The direct inhibition of GRK2 is a therapeutic target for the treatment of heart failure.(11) Studies in animal models with the GRK2 inhibitory protein, βARKct, or with cardiac-specific GRK2 gene deletion, have shown that inhibition of GRK2 or lowering its expression improves heart failure outcome.(12,13)

The selective Serotonin reuptake inhibitor paroxetine, which is approved as an antidepressant drug, specifically binds to the catalytic domain of GRK2 as an off-target activity. Myocardial contractility has been significantly potentiated by moderate concentrations of paroxetine in vitro and in vivo. In a mouse model, paroxetine-mediated GRK2 inhibition has been shown to reverse cardiac dysfunction and remodeling after myocardial infarction.(14) Serum levels of paroxetine in mice at 4 weeks were comparable to serum levels in humans treated for depression with paroxetine at dosages of 10 to 60 mg/day (27 to 192 ng/ml in mice versus 5-190 ng/ml in humans). Mice treated with paroxetine experienced a robust increase in left ventricular function of approximately 30%, and an absolute increase in fractional shortening of 20% at 6 weeks after myocardial infarction. Simultaneous treatment with the beta-blocker metoprolol showed a consistent effect of paroxetine, suggesting that the therapeutic translational potential of paroxetine may have an incremental benefit to the current standard of care. The objective of the present study is to investigate the off-target effect of paroxetine to reverse cardiac remodeling and improve left ventricular ejection fraction in patients after acute myocardial infarction.

3.2 Investigational Product and Indication

Paroxetine is a potent selective serotonin (5-hydroxytryptamine) reuptake inhibitor (SSRI); as an off-target effect binds to the catalytic domain of GRK2. Paroxetine is approved to treat major depression, obsessive-compulsive disorder, panic disorder, social anxiety, posttraumatic stress disorder, generalized anxiety disorder, and vasomotor symptoms associated with menopause in adult outpatients. Paroxetine is contraindicated in all patients under 18, and pregnant women. Adverse effects of paroxetine include nausea, diarrhea, constipation, dry mouth, somnolence, insomnia, headache, hypomania, blurred vision, weight gain, nervousness, paresthesia, dizziness, asthenia, tremor, sweating, and sexual dysfunction. Most of the side effects are self-limited and disappear with continued treatment. Interactions with MAOIs or antipsychotic or other dopamine antagonists may increase the risk for the potentially life-threatening neuroleptic malignant syndrome. (15-17)

3.3 Preclinical Evidence

Ventricular remodeling is an important determinant of cardiovascular outcome in survivors of acute myocardial infarctions and an important target for current treatment strategies.(18) Agents such as angiotensin-converting enzyme (ACE) inhibitors and beta-adrenergic blocking agents are known to significantly influence the remodeling process and translated into a reduction of morbidity and mortality.

An increased sympathetic drive to compensate for decreased cardiac output importantly contributes to the maladaptive changes characteristic of heart failure. The failing pump function promotes increased levels of circulating catecholamines, resulting in severe uncoupling of β-adrenoceptors and a loss of inotropic reserve. Dysregulation of G-protein coupled receptor (GPCR) is one of the pathophysiological pathways of heart failure progression through an increased adrenergic stimulus. Enhanced activation of GPCR kinases (GRKs) mediated by overstimulation of beta-adrenergic receptors (BAR) results in downregulation of BAR density, translating into a loss in inotropic reserve.(19,20)

A two to three fold in increase in GRK2 activity has been observed in failing human hearts, and is thought to have a central role in adverse cardiac remodeling after ischemic injury.(21) Competitive binding of a C-terminal peptide to Gβγ inhibits GRK2, and has been shown to prevent and reverse heart failure. The direct inhibition of GRK2 has been studied for several years and is a therapeutic target for the treatment of heart failure.(22,23) Studies in mice overexpressing GRK2 in the heart show attenuation of isoproterenol-stimulated contractility, reduced cAMP levels, and impaired cardiac function.(22) Studies in animal models with the GRK2 inhibitory protein, βARKct, or with cardiac-specific GRK2 gene deletion, have shown that inhibition of GRK2 or lowering its expression improves heart failure outcome.(12,24)

The selective Serotonin reuptake inhibitor paroxetine specifically binds to the catalytic domain of GRK2 as an off-target activity with selectivity over other GRK subfamilies. Myocardial contractility has been significantly potentiated by moderate concentrations of paroxetine in vitro and in vivo.(11) Paroxetine is approved as an antidepressant drug. GRK2 inhibition is specific for paroxetine and is not a class effect of other SSRIs such as fluoxetine. In mouse model, paroxetine-mediated GRK2 inhibition has been shown to reverse cardiac dysfunction and remodeling after myocardial infarction(14). Wild-type mice were started 2 weeks after induced myocardial infarction on paroxetine at a dosage of 5 mg/kg, dimethyl sulfoxide in water, or fluoxetine (5 mg/kg) over a course of 4 weeks. Serum levels of paroxetine in mice at 4 weeks were comparable to serum levels in humans treated for depression with paroxetine at dosages of 10 to 60 mg/day (27 to 192 ng/ml in mice versus 5-190 ng/ml in humans). Mice treated with paroxetine experienced a robust increase in left ventricular function of approximately 30%, and an absolute increase in fractional shortening of 20% at 6 weeks after myocardial infarction. Evidence from histopathological analysis indicated preservation of structural integrity of myocardium of mice treated with paroxetine. In order to corroborate that the improvement of left ventricular function was related to the inhibition of GRK2, the study was replicated with cardiac-targeted βARKct transgenic mice, as well as GRK2-overexpressing mice. A persistent effect of paroxetine-mediated GRK2-inhibition was demonstrated 2 weeks after cessation of paroxetine. Simultaneous treatment with the beta-blocker metoprolol showed a consistent effect of paroxetine, suggesting that the therapeutic translational potential of paroxetine may have an incremental benefit to the current standard of care.

3.4 Clinical Evidence to Date

There is no available clinical research data to date on paroxetine intake in terms of adverse cardiac remodeling.

3.5 Dose Rationale: Rationale for the intended purpose in study (pre-market MD)

Paroxetine or placebo will be started per os <7days of primary PCI after myocardial infarction for in total 13 weeks. Paroxetine will be given at a dose of 20mg once daily as it is commonly used in regular medical treatment for week 1-12. To stop abrupt discontinuation, Paroxetine will be given in a dose of 10mg in week 13.

3.6 Explanation for choice of comparator (or placebo)

All patients will receive standard medical therapy after myocardial infarction (in particular including ACE-inhibitors/AT1-AT2-antagonists and beta-blocker therapy). As this study does not target the comparison between drugs which influence cardiac remodeling a placebo will be used in the control group.

3.7 Risks / Benefits

Subjects enrolled in both the treatment and control arms of this study are required to have a decreased LVEF ≤45%. It is not anticipated that the treatment group will be at higher risk of experiencing one or more of the listed complications versus the control group. All adverse events will be closely monitored throughout the entire duration of the study.

3.7.1 Benefits

The potential benefits from this study fall into the following categories:

- Decreased left ventricular remodelling and improved left ventricular function in patients allocated to the treatment arm
- Improved exercise tolerance in patients allocated to the treatment arm
- Close clinical follow-up in the setting of the study protocol
- Possible benefits for future patients receiving the treatment based upon results of the proposed study.

3.7.2 Risks

As with any subject undergoing medical therapy with paroxetine, subjects in this study may experience adverse events and/or outcomes that may include, but are not necessarily limited to the following:

frequently:

- sleepiness
- increased cholesterol levels
- weakness / powerlessness
- dizziness / tremor
- headache
- nausea / gastrointestinal disorders
- sweating
- sexual dysfunction
- weight gain

rarely:

- bleeding of skin and/or mucosa
- skin rash / pruritus
- hyponatremia / seizure
- elevation of liver parameters
- confusion / agitation / hallucinations
- panic attacks / anxiety
- acute glaucoma
- bradycardie
- vasodilatation

- In patients with diabetes blood sugar levels may change and possibly the dose of treatment must be adapted
- increased risk of bone fracture
- limitations may arise while operating a motor vehicle and operating machinery

When issuing, the drug may cause dizziness, sensory disturbances, sleep disturbances, anxiety and headache. Occasionally restlessness, nausea, tremor, confusion, sweating, emotional instability, visual disturbances, palpitations, irritability and diarrhea.

To avoid abrupt discontinuation of the study drug, the study dosage will be decreased by 50% after 12 weeks for a time period of one week, afterwards it will be completely stopped.

In terms of cardiac MRI, no contrast medium will be used.

Patients will undergo thorough pre-procedural assessment prior to selection and inclusion into the study. Following randomisation, patients will be closely monitored by their physicians. Careful medical follow-up is required for detection and adequate management of potential complications.

3.8 Justification of choice of study population

The study population consists of patients with acute ST-segment elevation myocardial infarction as this is a cohort in which cardiac remodeling can be targeted and analyzed at its earliest stage after a known myocardial damage. We will confine eligibility to transmural anterior wall myocardial infarctions, since anterior wall myocardial infarction has been associated with the most extensive myocardial remodeling.(9,25)

4. STUDY OBJECTIVES

4.1 Overall Objective

The objective of this study is to evaluate whether paroxetine-mediated GRK2 inhibition reverses cardiac dysfunction and remodeling after acute anterior wall ST-segment elevation myocardial infarction.

4.2 Primary Objective

The primary objective is to evaluate whether paroxetine-mediated GRK-2 inhibition following myocardial infarction improves left ventricular ejection fraction over a course of 3 months as assessed by cardiac MRI (cMRI).

5. STUDY OUTCOMES

5.1 Primary Outcome

The primary endpoint will be the difference in the change (Δ) in LVEF at 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cMRI.

5.2 Secondary Outcomes

- in left left-ventricular end-diastolic volume (LVEDV) between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI.

- in left left-ventricular end-systolic volume (LVESV) between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI.

- in late-enhancement between baseline and 12 weeks in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by cardiac MRI.

- in LVEF between baseline and 12 weeks, and 12 months, respectively in subjects treated with paroxetine 20 mg QD or placebo QD as assessed by transthoracic echocardiography.

- major adverse cardiac events (cardiac death, myocardial infarction, repeat hospitalization for heart failure) at 12 weeks and 12 months, respectively.

- clinical symptoms of heart failure as assessed by New York Heart Association (NYHA) at 12 weeks and 12 months, respectively.

5.3 Safety Outcomes

Incidence and severity of side effects related to study drug intake throughout the entire study.

6. STUDY DESIGN

6.1 General study design and justification of design

A total of 50 patients presenting with acute myocardial infarction (<24 hours since symptom onset) for primary percutaneous coronary intervention will be randomized in a 1:1 ratio to treatment with paroxetine or placebo <7 days after primary PCI. Randomisation will be performed by the use of sequentially numbered, sealed, opaque, tamper-proof security envelopes. Study participants will be blinded to the treatment allocation. Patients will be treated with paroxetine 20 mg QD or placebo QD for the duration of 12 weeks in addition to standard medical therapy for myocardial infarction (in particular including ACE-inhibitors/AT1-AT2-antagonists and beta-blocker therapy). LVEF will be assessed at baseline and at 12 weeks by the use of transthoracic echocardiography and cardiac MRI. Paroxetine serum levels will be measured at 12 weeks. In week 13, a paroxetine dose of 10mg QD or placebo QD will be given to avoid abrupt discontinuation. Telephone follow-up will be performed at 1 month; clinical follow-up will be performed at 12 weeks, and at 12 months. The study design as well as the GANTT chart is illustrated in Appendix Figure 1 and 2.

6.2 Methods of minimising bias

6.2.1 Randomisation

Randomisation will be performed after primary percutaneous coronary intervention has been performed, all eligibility criteria have been checked, and written informed consent has been obtained. The allocation schedule is based on computer-generated random numbers and randomisation will be performed using RedCap software. Patients are assigned on a 1:1 basis to treatment with a paroxetine 20 mg q.d. for 12 weeks(week 1-12)/10mg q.d. for 1 week (week 13) or placebo q.d. for 13 weeks, respectively.

6.2.2 Blinding procedures

Placebo tablets specifically prepared for this trial will be identical to the real drug in color, appearance, smell and taste, as well as packaging and labeling. Trial participants, care providers, outcome assessors, and data analysists will be blinded to the assignment of treatment.

6.3 Unblinding Procedures (Code break)

In case of adverse events or serious adverse events, the Sponsor Investigator will be allowed during the trial to unblind the randomized substance. Randomisation codes will be stored via REDcap, hosted at the clinical trials unit, and can be unblinded within the same system. The principal investigator, Thomas Pilgrim and the co-investigator René Vollenbroich, will have access to this database, also in case of suspension or premature study termination.

7. STUDY POPULATION

A total of 50 patients will be enrolled at the University Hospital in Bern, Switzerland during a period of 6 months.

7.1 Eligibility criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

- Acute anterior wall ST-segment elevation myocardial infarction
- Informed Consent as documented by signature (Appendix Informed Consent Form)
- Age ≥18 years
- Primary PCI within 24 hours of symptom onset

- LVEF ≤ 45% within 48-96h after primary PCI (TTE)

The presence of any one of the following **exclusion criteria** will lead to exclusion of the participant:

- Contraindications to the SSRIs, e.g. known hypersensitivity or allergy to class of drugs or the investigational product
- Intolerance to Paroxetine
- Current medical therapy with MAO-blocker (during, 14 days before, and 14 days after treatment with MAO-blocker), lithium, thioridazide, or pimozide,
- Women at reproductive age (<50 years)
- Significant renal failure, hepatic dysfunction
- Known or suspected non-compliance, drug or alcohol abuse,
- Inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc. of the participant,
- Participation in another study with paroxetine within the 30 days preceding and during the present study,
- Previous enrolment into the current study,
- Enrolment of the investigator, his/her family members, employees and other dependent persons,
- *Current Paroxetine treatment*
- Previous myocardial infarction
- Previous revascularization procedure (percutaneous coronary intervention or coronary artery bypass grafting).
- Contraindications to CMR (metallic foreign bodies)

7.2 Recruitment and screening

Participants are recruited by a designated research fellow at the department of cardiology, University Hospital Bern, Switzerland. No specific screening requirements are necessary. Study participants will be compensated for the clinical follow-up visits (refunding costs of public transport).

7.3 Assignment to study groups

Assignment to study groups will be performed as described in 6.2.1.

7.4 Criteria for withdrawal / discontinuation of participants

In case of withdrawal of informed consent, non-compliance, or possible side-effects of the study drug participants will be withdrawn from the study.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment)

8.1.1 Experimental Intervention (treatment)

Paroxetine (Deroxat), a pill, which is taken per os.

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Placebo, a pill (same colour, same form as IP), which is taken per os.

8.1.3 Packaging, Labelling and Supply (re-supply)

Both paroxetine and placebo encapsulated tablets will be provided in identical boxes with identical labels; all study participants will receive the supply for the entire study duration of 12 + 1 weeks after randomisation, and will need no refills.

8.1.4 Storage Conditions

Paroxetine supplies is kept in a secure, limited access storage area under standard recommended storage conditions. Supply, storage, return or destruction are performed according to standard procedures.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

Paroxetine will be taken according to standard treatment procedures, per os, once daily.

8.2.2 Control Intervention

Placebo will be taken according to standard treatment procedures, per os, once daily.

8.3 Dose modifications

In case of adverse side effects of the study drug or placebo, intercurrent major cardiovascular event, or participant request, the drug will be discontinued.

8.4 Compliance with study intervention

A telephone follow-up at 1 month will be performed to confirm adherence to the study drug. Serum paroxetine level will be measured at 12 weeks in order to assess compliance. Study participants will be asked to return the empty bottles (with unused medication) at the follow-up visit at 12 weeks. Non-compliance will be documented in case of discontinuation or interruption of the study drug, or a serum level of <30 ng/ml at 12 weeks in patients allocated to paroxetine. Analysis of the primary endpoint will be intention-to-treat.

8.5 Data Collection and Follow-up for withdrawn participants

All data of withdrawn participants will be analyzed in the same way which will be used for participants. All data and material will be anonymized. No further follow-up for withdrawn patients is planned.

8.6 Trial specific preventive measures

Concomitant treatment with MAO-inhibtors or other SSRI's are disapproved during the first 12 weeks of the study. We expect no impact on the primary objective of our study from this measure.

8.7 Concomitant Interventions (treatments)

In line with the recent clinical evidence, we advocate complete revascularization at the time of primary PCI or during index hospitalization. However, we leave the final decision for culprit-only or complete revascularization to the discretion of the operator. All patients will receive standard medical regimen for secondary prevention after myocardial infarction. Furthermore, patients are free to enroll for an in-patient or out-patient cardiac rehabilitation program. We expect no impact on the primary objective of our study from this measure.

8.8 Study Drug / Medical Device Accountability

The University Hospital Pharmacy takes care of the production as well as of the disposal of the study drug as well as the placebo. Both the study drug as well as the placebo will be stored and appropriately secured. After the patients return the empty packages, remaining encapsulated tablets and/or packages will be destroyed according to the local standards. Return or Destruction of Study Drug / Medical Device

At the end of the study, the IP will be destroyed.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments

See Appendix No 1

9.2 Assessments of outcomes

9.2.1 Assessment of primary outcome

The primary outcome, change of LVEF (in %) will be measured at week 12. TTE will be used assessing LVEF with Simpson biplane method and/or visual estimation/Teichholz estimation. In addition LVEF will be measured with cardiac MRI. For both methods, patients should be in a supine or left-sided position for at least 15 minutes.

9.2.2 Assessment of secondary outcomes

The secondary outcomes, LVEF change (in %) will be measured at 12 months. As the primary outcome TTE (but no cardiac MRI) will be used assessing LVEF with Simpson biplane method and/or visual estimation/Teichholz estimation.

9.2.3 Assessment of other outcomes of interest

Adverse event information will be assessed at 1 month, 3 months, and 12 months.

9.2.4 Assessment of safety outcomes

9.2.4.1 Adverse events

The following adverse event information will be recorded: time of onset, duration, resolution, action to be taken, assessment of intensity, relationship with study treatment; definition of AE and procedures are outlined in Section 10. We will inquire for the occurrence of adverse events during each follow-up contact using structured interviews. Spontaneous reports will be collected from primary care physicians and referring hospitals.

9.2.4.2 Vital signs

Heart rate, blood pressure, body temperature, oxygen saturation, respiratory rate, and ECG will be performed at each follow-up visit and require a supine position after 5 minutes resting.

9.2.5 Assessments in participants who prematurely stop the study

Participants who prematurely stop the study will be asked to participate in the regular follow-ups including all planned clinical and procedural steps.

9.3 Procedures at each visit

9.3.1 Screening Visit (Day 0-1)

- Patient Information and Informed Consent
- Demographics
- Medical History
- In-/Exclusion Criteria
- Physical Examination
- Vital Signs
- Laboratory Tests
- ECG

9.3.2 Visit 1 (Day 2-6)

- Vital Signs
- TTE
- Cardiac MRI

9.3.3 Visit 2 (<7days)

- Randomisation depending on TTE/MRI results
- Administer Study Medication/Placebo
- Scheduling next follow-up

9.3.4 Visit 3 (4 weeks)

- Phone Follow-Up

9.3.5 Visit 3 (12 weeks)

- TTE
- Cardiac MRI
- Assessment of serum level of paroxetine
- Physical examination
- Vital Signs
- ECG
- Laboratory Tests
- Scheduling next follow-up

9.3.6 Visit 4 (12 months)

- TTE
- Physical examination
- Vital Signs
- ECG

10. SAFETY

10.1 Drug studies

The Sponsor's SOPs provide more detail on safety reporting.

During the entire duration of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and case report forms (CRF). Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A **Serious Adverse Event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs are followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease after termination.

Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out

*Improvement after dechallenge only taken into consideration, if applicable to reaction

Unexpected Adverse Drug Reaction

An “unexpected” adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator’s Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR. In order to determine a SUSAR unblinding will be performed.

Assessment of Severity

The Common Terminology Criteria for Adverse Events CTCAE Version 4.03 will be used for assessing the grade of severity.

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

All SAEs are reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study. The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.

SAEs resulting in death are reported to the local Ethics Committee (via local Investigator) within 7 days.

Reporting of SUSARs

A SUSAR will be reported to the local Ethics Committee (local event via local Investigator) and to Swissmedic within 7 days, if the event is fatal, or within 15 days (all other events).

Reporting of Safety Signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures (safety signals) will be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator will report the safety signals within 7 days to the local Ethics Committee (local event via local Investigator) and to Swissmedic.

Periodic reporting of safety

An annual safety report is submitted once a year to the local Ethics Committee via local Investigator and to Swissmedic.

10.1.3 Follow up of (Serious) Adverse Events

The follow-up of participants terminating the study (either regularly or prematurely) with reported ongoing SAE, or any ongoing AEs of laboratory values or of vital signs being beyond the alert will be performed until the adverse event resolves or until a stable clinical endpoint is reached.

11. STATISTICAL METHODS

11.1 Hypothesis

We formulate the following Null Hypothesis: "There is no difference in LVEF recovery between patients with acute ST-segment elevation myocardial infarction with or without administration of Paroxetine, respectively". Our Alternative Hypothesis is thus as follows: "The administration of Paroxetine in patients with acute ST-segment elevation myocardial infarction increases LVEF by 10% compared to the control group". Using this hypothesis we will be able to evaluate the off-target effect of Paroxetine on cardiac remodeling.

11.2 Determination of Sample Size

For each arm of the study the estimated number of participants will be 25. We assume a difference in delta LVEF of 10% in the treatment group compared to the control group with a standard deviation of 10% (increase of 5% in the control group and 15% in the treatment group) after 12 weeks. Dropout rate at 12 weeks was estimated to be 10% in both groups. We calculated a sample size of 50 patients to provide more than 90% power to detect superiority on the primary endpoint at a two-sided type I error of 0.05.

The difference in delta LVEF of 10% was assumed aligning with the study results of Schumacher et al. in which they found even an absolute increase in LVEF of 30% after the intake of Paroxetin (14).

11.3 Planned Analyses

11.3.1 Primary Analysis

This is an exploratory trial with an intention-to-treat analysis design for the primary outcome. Continuous variables will be summarised by means and standard deviations (SD) for normally distributed continuous variables or medians and interquartile ranges otherwise. Categorical variables will be summarised by frequencies and percentages. P-values will be computed using Chi-square or (in case of few events) Fisher's tests for categorical variables, Student's t-test for normally distributed continuous variables and Wilcoxon's Mann-Whitney U-test for non- symmetrically distributed continuous variables. The p-values will refer to differences across groups at patient-level. All tests will be two-sided and a p-value <0.05 will be considered statistically significant.

Data is planned to be available by September 2017 and will be analyzed by CTU Bern in the following six months.

11.3.2 Secondary Analyses

The sample size does not qualify for secondary analyses.

11.3.3 Interim analyses

No interim analyses are planned.

11.3.4 Deviation(s) from the original statistical plan

As this is an exploratory study with a small sample size allowing only for descriptive statistics, we are not expecting deviations from the original statistical plan

11.4 Handling of missing data and drop-outs

All analyses are based on the intention-to-treat as this is a randomized clinical trial. No multiple imputation techniques are needed for handling of missing data. The sample size calculation accounted for an estimated drop-out rate of 10% after 12 months. The primary outcome will be analysed on the last valid contact date with each patient.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

All data will be recorded in electronic case report forms in REDCap, hosted at the clinical trials unit. All study related documents (essential documents and site documents) are archived.

12.1.1 Case Report Forms

Study data will be recorded with electronic case report forms. For each enrolled study participant, a CRF is maintained. Appropriate coded identification with a specific participant number in combination with the year of birth will be used so that anonymity can be guaranteed. CRFs will neither include names nor participants initials. Study nurses form the study team in Bern will be authorized for all CRF entries. Any authorized person can be identified.

12.1.2 Specification of source documents

Source data is available at the site to document the existence of the study participants. Source data include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

Demographic data, visit dates, participation in study and Informed Consent Forms, randomisation number, SAEs, AEs and concomitant medication, and results of relevant examinations are considered source documents.

12.1.3 Record keeping / archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial at the department of cardiology of the University of Bern, Switzerland.

12.2 Data management

12.2.1 Data Management System

REDCap electronic data entry system will be used, which is hosted at the clinical trials unit of the University of Bern, Switzerland. Martina Rothenbühler from clinical trials unit will be responsible for database management.

12.2.2 Data security, access and back-up

The principal investigator, the co-investigators, and the responsible biostatistician, as well as the study coordinator will have access to the electronic database throughout the course of the study. A local backup of the electronic database will be downloaded once a week.

12.2.3 Analysis and archiving

Data from REDCap will be downloaded to STATA for statistical analysis. The data will be stored in REDCap for the duration of 10 years after completion of the study. MRI and echocardiographic data will be stored as usual in the clinical setting. Blood samples will be destroyed according to local standards.

12.2.4 Electronic and central data validation

Central data monitoring will be performed by the biostatistician. In case of inconsistencies, data will be verified using source data documentation.

12.3 Monitoring

Central data base monitoring will be performed by the biostatistician in order to detect inconsistencies in data recording. No monitoring of source data will be performed.

12.4 Audits and Inspections

The study documentation and the source data/documents are accessible to auditors/inspectors (also CEC and CA) and questions are answered during inspections. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

Direct access to source documents will be permitted for purposes of monitoring (12.3), audits and inspections (12.4) (ICHE6, 6.10). The principal investigator (TP), the co-investigators (LH, RV), and the biostatistician will have access to protocol, dataset, and statistical code during and after the study (publication, dissemination).

13. PUBLICATION AND DISSEMINATION POLICY

All publications will follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by the International Committee of Medical Journal Editors

14. FUNDING AND SUPPORT

14.1 Funding

The study will be funded through a CTU (clinical trials unit) grant of the University Hospital Bern.

14.2 Other Support

There will be no other support.

15. INSURANCE

Insurance will be provided by the Sponsor (Zürich Versicherungs-Gesellschaft AG). A copy of the certificate is filed in each investigator site file and the trial master file.

16. REFERENCES

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17. APPENDICES

Figure 1: Study Flow Chart

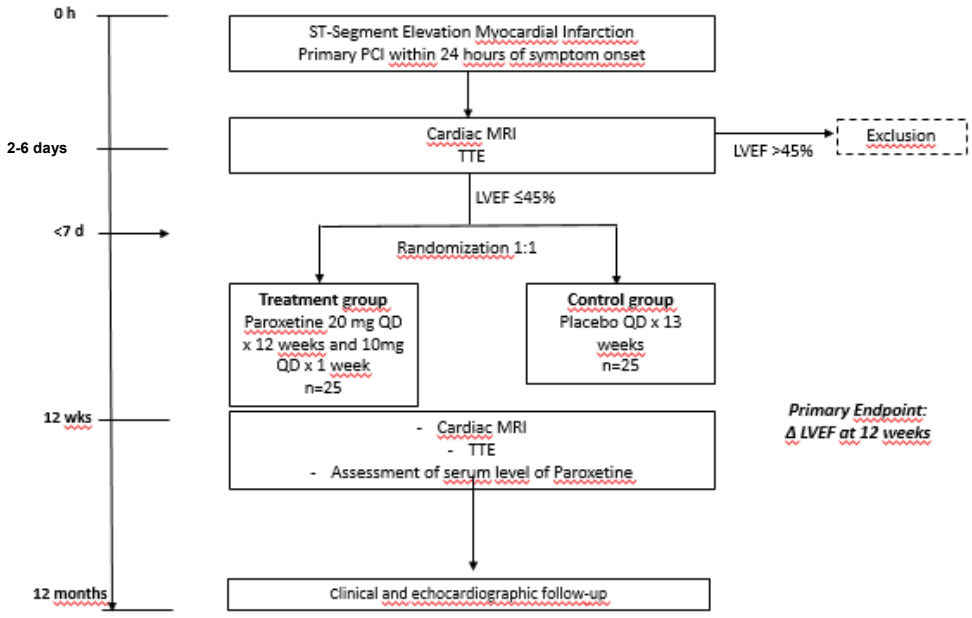
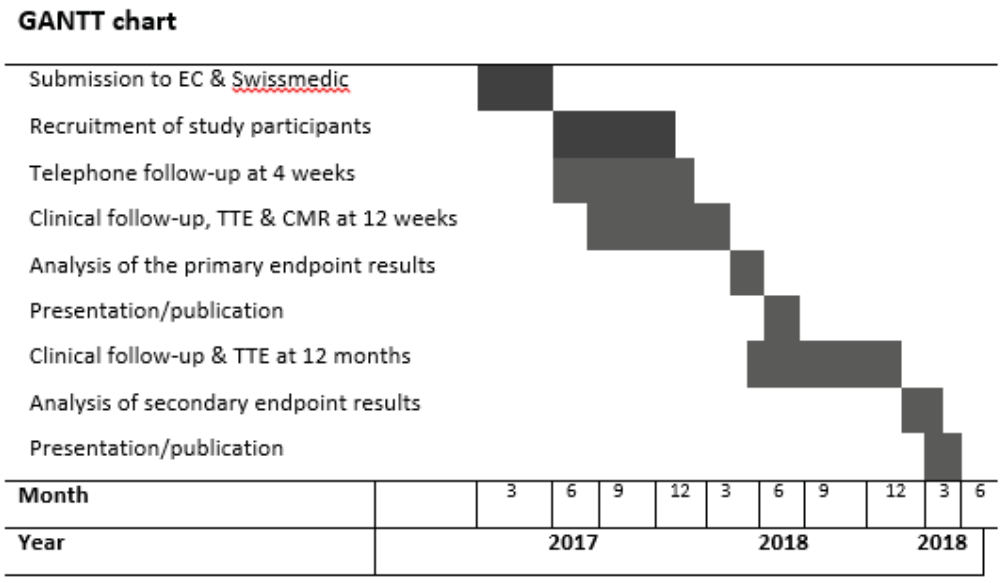


Figure 2: GANTT chart showing the timeline and major milestones CARE-AMI study



Attachment 1.1: Case Report Form Screening
Attachment 1.2: Case Report Form 4 weeks
Attachment 1.3: Case Report Form 12 weeks
Attachment 1.4: Case Report Form 12 months