GENERAL INFORMATIONS

1. Title: PROgnostic value of precision medicine in patients with Myocardial Infarction and non-obStructive coronary artEries: the PROMISE study.

2. Trial Registration: The study will be registered on ClinicalTrials.gov, and the study page updated with the study results if possible.

3. Version*:* 3_06_May_2021

4. Founding: Co-founded study (project " Ricerca Finalizzata 2019", Ministry of Health)

5.a *Principal Investigator (PI) of the Promoter Center:*

Rocco Antonio Montone [1] (*roccoantonio.montone@policlinicogemelli.it*) , MD, PhD
Department of Cardiovascular Medicine
Intensive Cardiology Unit
Fondazione Policlinico Universitario A. Gemelli IRCCS
L.go A. Gemelli, 1 – 00168 Rome, Italy
Tel. +39-06-30154187
Fax. +39-06-3055535

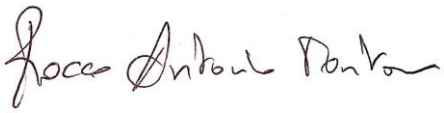
Co-PI of the Promoter Center: Dr. Francesca Graziani[1] (*francesca.graziani@policlinicogemelli.it*)

[1] Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italia.

5b-c. Sponsor: Italian Ministry of Health

5d. The PIs of the participating centers are responsible for conducting the clinical trial in accordance with the ICH-E6 guidelines and the GCP rules. They are involved in the design and planning of the clinical trial and are responsible for all trial-related medical decisions at their clinical site. Furthermore, they are responsible for training all staff involved in the conduct of the trial at their site and for reporting all adverse effects (SAEs) to the Investigator. The authors of the protocol are solely responsible for the collection, management, analysis and interpretation of the data; in writing reports and deciding to submit reports for publication.

Date: May 6, 2021 **Signature**



6. Abstract

Background: Myocardial infarction with non-obstructive coronary arteries (MINOCA) represents about 6-8% of patients presenting with myocardial infarction. Initially believed to be a benign condition, is now becoming clear that MINOCA is associated with a significant risk of mortality, rehospitalization, disability and angina burden, with high socioeconomic costs. However, to date there are no prospective clinical trials in this population and treatment of these patients is not still defined, also because there are multiple pathogenic mechanisms underlying MINOCA.

Objectives: The aim of our study is to evaluate if the use of a precision-medicine approach with a specific therapy tailored on the underlying pathogenic mechanism will improve the quality-of-life in MINOCA patients *(primary objective)*. We further aim at investigating wherever a precision-medicine approach will improve the prognosis, healthcare related costs, and if that a different profile of plasma biomarkers and microRNAs may serve as diagnostic tools for detecting specific causes of MINOCA and to assess response to therapy (*secondary objectives*). Finally, beyond its pivotal role in differential diagnosis, we hypothesize that cardiac magnetic resonance (CMR) may provide a morphological and functional cardiac characterization as well as help in the prognostic stratification (*secondary objective).*

Methods: We will include 180 patients aged >18 years hospitalized for MINOCA randomized 1:1 to a "precision medicine approach" consisting of a comprehensive diagnostic work-up, analysis of circulating biomarkers and micro RNA expression profile and pharmacological treatment specific for the underlying cause *versus* a "standard approach" consisting of routine diagnostic work-up and standard medical treatment.

INTRODUCTION

6.a Randomized multicenter prospective superiority phase IV trial comparing "precision medicine approach" *versus* "standard of care" in improving the prognosis and/or the quality-of-life of patients presenting with MINOCA. Patients will be randomized 1:1 to "precision medicine approach" consisting of a comprehensive diagnostic work up aim at elucidating the pathophysiological mechanism of MINOCA and consequently a tailored pharmacological approach *versus* "standard of care" consisting of standard diagnostic algorithm and therapy for myocardial infarction.

6.b Background: Myocardial infarction with non-obstructive coronary arteries (MINOCA) represents about 6-8% of all patients presenting with acute myocardial infarction (MI) referred for coronary angiography. MINOCA is defined by the evidence of MI with normal or near normal coronary arteries on angiography, in absence of a specific alternate diagnosis for the clinical presentation (i.e. sepsis, myocarditis, pulmonary embolism). Notably, there is a variety of causes underlying MINOCA including coronary plaque rupture/erosion, epicardial or microvascular spasm, and coronary embolism. Therefore, MINOCA should not be considered as a single entity but a heterogeneous working diagnosis that requires a comprehensive evaluation to elucidate the potential underlying cause. Several studies have shown that MINOCA patients have 1-year mortality and rehospitalization rate similar to those patients with acute MI with obstructive coronary artery disease (MICAD). Furthermore, approximately 25% of patients with MINOCA will experience angina in the subsequent 12 months, at least as high as reported in patients with MICAD, with a significant impact on quality of life and healthcare related costs. Angina symptoms have also relevant socioeconomics consequences because patients with angina without evidence of CAD have the same increased probability of future disability pension and premature exit from the workforce as patients with obstructive CAD. This is of crucial importance, because MINOCA patients are usually younger than patients with MICAD. A comprehensive evaluation and a multimodality assessment aiming at uncovering the aetiology should be pursued, in order to implement tailored therapeutic approaches targeted to the specific underlying cause. Indeed, the management of MICAD has well-established evidence-based guidelines, while the management of MINOCA has a limited evidence-based literature, with no prospective randomized controlled trials undertaken to date. In particular, a precision medicine approach is important in MINOCA patients because of the multiple causes underlying this condition. Importantly, a precision-medicine approach had already proved its efficacy

in patients with stable angina and non-obstructive CAD. Indeed, the landmark Coronary Microvascular Angina (CorMicA) trial showed that in these patients a strategy of adjunctive invasive testing for disorders of coronary function linked with stratified medical therapy led to improvement in patient outcomes, including reduction in angina severity and better quality of life. If a similar precision-medicine approach is effective also in patients with MINOCA has never been assessed. We previously demonstrated that patients with a positive provocative test for epicardial or microvascular spam had a higher occurrence of future cardiac events, thus identifying high-risk MINOCA patients that may need a more aggressive therapy. Moreover, biochemical profile of MINOCA patients is still largely unknown and whatever exist a unique profile associated with specific pathophysiological mechanisms remains unknown. Endothelin-1 (ET-1) and neuropeptide Y (NPY) are endogenous vasoactive substances regulating coronary vasomotion involved in coronary spasm. On the other hand, elevated plasma levels of soluble CD40L and C-reactive protein (CRP) are involved in inflammatory activation during MI with plaque rupture. Moreover, also circulating miRNA may be associated with specific pathophysiological mechanisms of MINOCA, and may represent new promising diagnostic tools. Particularly, the up-regulation of miR-16 and miR-26a and the down-regulation of miR-1 and miR-133, have been reported to characterize coronary microvascular spasm, whereas, miR-145 and miR-222 may help in identifying patients with epicardial spasm. Finally, miR-155-5p, miR-483-5p and miR-451a have been suggested as novel biomarkers for the early identification of plaque rupture. However, miRNA assessment in MINOCA patients has never been performed. In the absence of dedicated randomized controlled trials, current guidelines do not specifically address the issue about acute and long-term management of patients with MINOCA. The effects of secondary preventive treatments proven beneficial in patients with MICAD are unknown in MINOCA patients. The "one-size-fits-all" approach used for MICAD treatment may not apply uniformly to all MINOCA patients, which instead should be "personalized" depending on the underlying pathophysiological mechanism responsible for the clinical presentation. Thus, the next key step in the management of MINOCA is to demonstrate the benefits of tailored therapies on cardiovascular and quality-of-life outcomes. With this prospectively designed trial, we have the opportunity to test the prognostic value of a targeted therapeutic approach based on the identification of the underlying cause. Of importance, the results deriving from our trial may open the way for a new pathophysiology-driven approach with cause-target therapies personalized for the mechanisms of MINOCA.

6.c There is a limited agreement regarding the acute and long-term medical management of patients with MINOCA. The results of a large observational study of MINOCA patients from the

SWEDEHEART registry have shown a significantly lower rate of cardiovascular events associated with the use of statins and ACEi/ARB, a trend for a lower event rate with the use of ß- blockers, while dual antiplatelet therapy (DAPT), a cornerstone of therapy for MICAD patients, failed to improve prognosis. An important limitation of this study is the heterogeneous nature of MINOCA cohort, without discerning the mechanism leading to myocardial infarction. Given these therapeutic shortcomings in this evolving area of clinical research, multicentre randomized clinical trials are needed to determine the optimal medical therapy for MINOCA based on the specific cause of the syndrome. Importantly, a precision-medicine approach had already proved its effectiveness in patients with stable angina and/or signs of ischemia but no obstructive coronary artery disease (INOCA). Indeed, the CorMicA trial showed that in patients with INOCA a strategy of adjunctive invasive testing for disorders of coronary function linked with stratified medical therapy led to improvements in patient outcomes, including reduction in angina severity and better quality of life. Similarly, the identification of functional alterations of coronary circulation is relevant in the clinical setting of MINOCA. We previously demonstrated that MINOCA patients with a positive test result (epicardial or microvascular spam) had a significantly higher occurrence of cardiovascular events, thus identifying a high-risk subset of patients that may need a more aggressive therapy and a closer follow-up.

Moreover, information about cardiac structure and function as well as plasma biochemical profile associated with the different causes of MINOCA is scantly. Beyond its role in differential diagnosis, CMR may help in the morphological and functional cardiac characterization as well as in the prognostic stratification. Biochemical profile of patients with MINOCA is still unidentified and whatever exist a unique profile associated with specific pathophysiological mechanisms remains unknown. Endothelin-1 (ET-1) and neuropeptide Y (NPY) are endogenous vasoactive substances regulating coronary vasomotion, with higher circulating levels in patients with coronary microvascular and epicardial spasm. Elevated plasma levels of soluble CD40L and CRP, two inflammatory biomarkers associated with inflammation and thrombus formation, have been described in patients with MI due to acute plaque destabilization. Recent findings have shown that levels of specific circulating miRNA may have a close association with specific pathophysiological mechanisms of myocardial infarction (i.e. plaque rupture, microvascular spam or epicardial spasm). Particularly, a unique signature, comprising the up- regulation of miR-16 and miR-26a and the down-regulation of miR-1 and miR-133, has been reported to characterize coronary microvascular spasm, whereas miR-145 and miR-222 may help to identify patients with epicardial spasm. Finally, a recent study identified miR-155-5p, miR-483-5p and miR-451a, as novel biomarkers for the early

identification of plaque rupture. However, the role of circulating plasma biomarkers and miRNA in MINOCA patients has never been investigated.

7. Study objectives:

Primary objectives:

- To test if a precision-medicine approach with a careful investigation of the MINOCA aetiology and consequent aetiology-based treatment may result in improved quality of life outcomes.

Secondary objectives:

- To evaluate if the use of a precision-medicine approach with a specific therapy tailored on the MINOCA pathogenic mechanism will improve the prognosis.
- To assess if a precision-medicine approach with a careful investigation of the MINOCA aetiology and consequent aetiology-based treatment may result in reducing healthcare related costs.
- To test wherever a plasma circulating biomarker profile may be able to differentiate between the different causes of MINOCA, helping to identify the underlying pathophysiological mechanisms and to stratify prognosis and response to medical therapy.
- To test wherever cardiac magnetic resonance (CMR) may provide a morphological and functional cardiac characterization as well as help in the prognostic stratification in MINOCA.

8. Study design: Randomized multicenter prospective superiority phase IV trial comparing "precision medicine approach" *versus* "standard of care" in improving the prognosis and/or the quality-of-life of patients presenting with MINOCA. Patients will be randomized 1:1 to "precision medicine approach" vs "standard approach".

METHODS: PARTECIPANTS, INTERVENTIONS, OUTCOMES

9. Setting: IRCCS Fondazione Policlinico Universitario A. Gemelli (Promoter Coordinating Centre), Centro Cardiologico Monzino IRCCS, IRCCS Policlinico San Donato

Number of patients enrolled: The study will include 180 patients (expected 60 patients per center, but enrolment is competitive) aged >18 years hospitalized for MINOCA.

10. Inclusion criteria:

- Ability to give informed consent to the study
- Age > 18y
- MINOCA diagnosis, defined as:
    - Acute myocardial infarction (based on Fourth Universal Definition of Myocardial Infarction Criteria):
    - Evidence of non-obstructive coronary artery disease on angiography (i.e., no coronary artery stenosis >50%) in any major epicardial vessel.
    - No specific alternate diagnosis for the clinical presentation (i.e. non-ischemic causes of myocardial injury such as sepsis, pulmonary embolism, and myocarditis).

Exclusion criteria:

- Inability or limited capacity to give informed consent to the study
- Age < 18 y
- Pregnant and breast-feeding women or patients considering becoming pregnant during the study period will be excluded. For women of childbearing potential, the use of a highly effective contraceptive measure is required in order to be included in the study. “Highly effective contraceptive” is defined in accordance with the recommendations of the Clinical Trial Facilitation Group as a contraceptive measure with a failure rate of less than 1% per year (https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf).
- Alternate diagnosis for the clinical presentation (i.e. non-ischemic causes of myocardial injury such as sepsis, pulmonary embolism, valve disease, hypertrophic cardiomyopathy and myocarditis). Also patients presenting with Takotsubo syndrome will be excluded.

- Contraindication to contrast-enhanced CMR, eg, severe renal dysfunction (glomerular filtration rate <30 mL/min), non–CMR-compatible pacemaker or defibrillator.
- Contraindication to drugs administrated: e.g a history of hypersensitivity to drugs administrated or its excipients, significant renal and/or hepatic disease.
- Patients with comorbidities having an expected survival <1-year will be excluded.

Withdrawal criteria :

Subjects may be withdrawn from the study at discretion of the Investigator if one of the following criteria applies:

- Patients voluntary to stop participate to the study protocol.
- Progression of patient's disease or need to change medical therapy.
- Intolerable adverse effects.Pregnancy or intention of becoming pregnant at any time of the study.. Patients enrolled will have to communicate to the investigator if they get pregnant at any time of the study to be excluded by the study protocol.

Stopping rules

Premature termination of the clinical trial could be considered if the sponsor believes that it is necessary to terminate the clinical trial as a result of the observed efficacy of the treatment (benefit), of a likelihood of failing to reject the null hypothesis (futility), evidence of severe adverse effects of treatments administrated (harm) or when the clinical trial proved to be impracticable (e.g difficulty in patients enrolment).

11a. Intervention(s):

Patients with myocardial infarction undergoing to clinically indicated coronary angiography will be asked to sign the informed consent to the study before the coronary angiography/ventriculography and enrolled only after MINOCA diagnosis. MINOCA patients will be then randomized 1:1 (using an online software available 24h/24h) to:

A. "Precision medicine approach" consisting of:
Comprehensive diagnostic work-up with:

- Coronary angiography and ventriculography in all patients
- OCT (if coronary plaque rupture/erosion is suspected) at the time of coronary angiography in the cath-lab.
- Acetylcholine provocative test (to assess the presence of coronary vasospasm) at the time of coronary angiography in the cath-lab.
- TE-Echo and/or CE-Echo (if distal/microvascular embolization is suspected) during the hospitalization at the echo laboratory
- Blood sampling for circulating biomarkers and miRNA expression profile at the time of coronary angiography or within 12 hours from coronary angiography. Blood sampling will be processed and analysed in the research laboratory of the Department of Cardiovascular Science. Biological aliquots will be preserved at XBiogem Biobank at Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome.
- Trans-thoracic echocardiography in all patients during the index hospitalization
- CMR in all cases during the index hospitalization.

Targeted pharmacological treatment specific for the underlying cause:

- DAPT ± stent implantation (if required), statins, beta-blockers, ACEi/ARB (in case of evidence of plaque rupture/erosion)
- CCB and/or nitrates (in case of documentation of coronary vasospasm)
- Anticoagulation (in case of coronary embolism).

B. "Standard approach" consisting of:
Routine diagnostic work-up with:

- Coronary angiography and ventriculography
- Transthoracic echocardiography in all patients during the index hospitalization
- CMR with contrast media only if clinically indicated (i.e. to exclude myocarditis or takotsubo syndrome)

Standard medical treatment with:

- DAPT in all patients
- Beta-blockers (if indicated by the clinical context, i.e. documentation of left ventricular ejection fraction <50%, tachycardia).

- High intensity statins in all patients
- ACEi/ARB (if clinically indicated).

Pharmacological strategy will be started after the coronary angiography during the index hospitalization. All the procedures will be done by cardiologists with experience in the specific field. All three enrolling centers already have a high proficiency in performing all the procedures required for this study.

Interventional Procedures:

- *Coronary angiography*: coronary angiography will be performed via the transradial or transfemoral approach with the use of a 6F sheath. Coronary angiography will be performed within 90 minutes from hospital admission in patients presenting with persistent ST-segment elevation, and within 48 hours in patients presenting with non-ST-segment elevation. Unfractionated heparin (initial weight-adjusted intravenous bolus of 60 IU/Kg, with repeat boluses to achieve an activated clotting time of 250 to 300 seconds) was administered in all patients. If evidence of plaque rupture
- *Percutaneous coronary intervention (PCI)*: PCI with stent implantation will be considered in selected cases with evidences of plaque rupture
- *OCT imaging:* OCT imaging will be performed in the culprit artery in all patients randomized to the "precision medicine approach" with angiographic aspect of complex atheromatous plaque suggestive of plaque rupture. A 0.014-inch guidewire will be placed distally in the target vessel and an intracoronary injection of 200 μg of nitroglycerine will be performed. Frequency domain OCT (FD-OCT) images are acquired by a commercially available system (C7 System, LightLab Imaging Inc/ St Jude Medical, Westford, MA) connected to an OCT catheter (C7 Dragonfly; LightLab Imaging Inc/ St Jude Medical, Westford, MA), which was advanced to the culprit lesion. The FD-OCT run will be performed using the integrated automated pullback device at 20 mm/s. During image acquisition, coronary blood flow will be replaced by continuous flushing of contrast media directly from the guiding catheter at a rate of 4 ml/s with a power injector in order to create a virtually blood-free environment. Plaque rupture will be defined by the presence of fibrous cap discontinuity with a clear communication between the lumen and inner core of a plaque or with a cavity formation within the plaque. Plaque erosion will be identified by the presence of attached thrombus overlying an intact and visualized plaque, luminal surface irregularity at the culprit lesion in

the absence of thrombus, or attenuation of underlying plaque by thrombus without superficial lipid or calcification immediately proximal or distal to the site of thrombus. Considering that OCT does not provide an adequate resolution to identify the endothelial lining, the definition of plaque erosion should be considered a diagnosis of exclusion, requiring the absence of fibrous cap rupture. Thrombus will be defined as an irregular mass (diameter > 250 μm) either attached to the luminal surface or floating within the lumen.

- *TT-Echocardiography*: TT-Echo will be used to calculate left and right ventricular and atrial dimensions, left and right ventricular systolic function, transmitral flow Doppler spectra, mitral and tricuspidal valve annulus tissue Doppler spectra, ejection time and stroke volume, inferior vena cava, aorta and pulmonary artery diameters and Doppler spectra, according to the recommendations of the American Society of Echocardiography.
- *TE/contrast echocardiography:* In patients with angiographic evidence of distal microembolization, TE-Echo consisting of an echocardiographic probe inserted in to the oesophagus will be used to detect a hidden cardioembolic source (i.e. left atrial thrombus); in patients with suspected left ventricular source of cardioembolism, contrast echocardiography consisting of a 0.3ml solution of SONOVUE will be used.
- *Acetylcholine provocative test:* ACh will be administered in a stepwise manner into the left coronary artery (LCA) (20–200 μg) or into the right coronary artery (RCA) (20–50 μg) over a period of 3 min with a 2–3 min interval between injections. Coronary angiography will be performed 1 min after each injection of these agents and/or when chest pain and/or ischaemic ECG shifts were observed. The decision of testing with provocative test LCA or RCA as first will be left to the discretion of the physicians; both LCA and RCA will be tested if the first test was negative. Angiographic responses during the provocative test will be assessed in multiple orthogonal views in order to detect the most severe narrowing and/or analysed by using computerized quantitative coronary angiography (QCA-CMS, Version 6.0, Medis-Software, Leiden, The Netherlands).
- *Cardiac magnetic resonance:* CMR will be performed during hospital stay on a 1.5-T system equipped with a 32-channel cardiac coil. Patients underwent conventional CMR including cine, T2-weighted, first pass perfusion, and conventional breath-held late gadolinium enhancement (LGE).
- *Circulating biomarkers:* Blood sampling for circulating biomarkers and miRNA expression profile at the time or within 12 hours of coronary angiography. Blood sampling will be processed and analysed in the research laboratory of the Department of Cardiovascular

Science. Biological aliquots will be preserved at XBiogem Biobank at Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome (see section 33).

Risk analysis, possible problems and solutions:

- OCT is a safe invasive intracoronary imaging tool. Complications during manipulation inside coronary arteries are rare. This procedure will be performed by experienced operators to improve its safety.
- CMR with intravenous gadolinium administration is a safe procedure, associated with a low risk of renal damage, also in patients with severe kidney disease, and allergic reactions. These side effects may, however, be prevented and treated appropriately.
- A transesophageal echocardiogram (TEE) uses echocardiography to assess the structure and function of the heart. It is associated with a rare risk of complication, such as bleeding, airway difficulty, arrhythmias and local trauma. The risk of complications will be reduced by using an experienced sonographer and by excluding patients with Echo TE contraindications (oesophageal varices, oesophageal obstruction or stricture, or radiation therapy to the area of the esophagus). Contrast echocardiography refers to diagnostic ultrasound of the heart that is performed in conjunction with any acoustically active particle, including agitated saline and is not associated with any complication.
- Acetylcholine provocative test with intracoronary acetylcholine administration is associated with a very low risk of ischemic and/or arrhythmic events. However, recent studies confirmed that this test is safe and complications are rare, also in patients admitted with a diagnosis of MINOCA. Our center published a recent paper demonstrating the safety of this procedure and its prognostic relevance in MINOCA patients (see references). Occurrence of bradyarrhythmias (defined as bradycardia with heart rate < 50 bpm or second- or third-degree AV block lasting more than 3 s), atrial fibrillation and ventricular tachycardia (defined as three or more consecutive premature ventricular complexes) during the provocative are rare and if complications occur, patient will be treated appropriately with vasodilator pharmacological agents able to counteract the pharmacological effect of acetylcholine.

Pharmacological interventions:

All the drugs used for this trial are already widely used in cardiology clinical practice and will follow the AIFA indications for which the specific medication has been approved (see RCP attached). All

the study drugs will be stored according to GCP in the center's experimental pharmacy and collected at the time of use.

- Dual antiplatelet therapy: acetylsalicylic acid (loading dose 250mg intravenously followed by 75mg orally) + P2Y12 receptor inhibitor (i.e. Clopidogrel, 300 or 600mg loading dose orally, followed by 75 mg orally daily).
- Statins (i.e. atorvastatin; dosages titrated on the patient's clinical characteristics)
- Beta-blockers (i.e. bisoprolol; dosages titrated on blood pressure, ECG, heart rate)
- ACEi/ARB (i.e. ramipril; dosages titrated on blood pressure, ECG, heart rate)
- CCB (i.e. diltiazem; dosages titrated on blood pressure, ECG, heart rate) and/or nitrates (nitroglycerine; dosages titrated on blood pressure, ECG, heart rate)
- Anticoagulation (i.e. warfarin; the selection of the anticoagulant agent will be based on the clinical scenario, contraindications etc.)

Patients could receive any of the medications belonging to the same drug class.

11.b The dosage of the drugs will be modified following adverse events, at the request of the participants, in presence of an improvement or worsening of the disease.

11.c There are no concomitant medications contraindicated. For each patient concomitant medications will be recorded at baseline and at any time of follow up. Moreover, the presence of interactions between drugs administrated or concomitant medications which may influence the outcome of the study will be evaluated by the investigator at baseline and at any time of follow up".

12. Outcomes of interests:

Primary endpoints:

- Angina status and quality of life (evaluated using the Seattle Angina Questionnaire [SAQ]) at 1-year follow-up in patients with MINOCA.

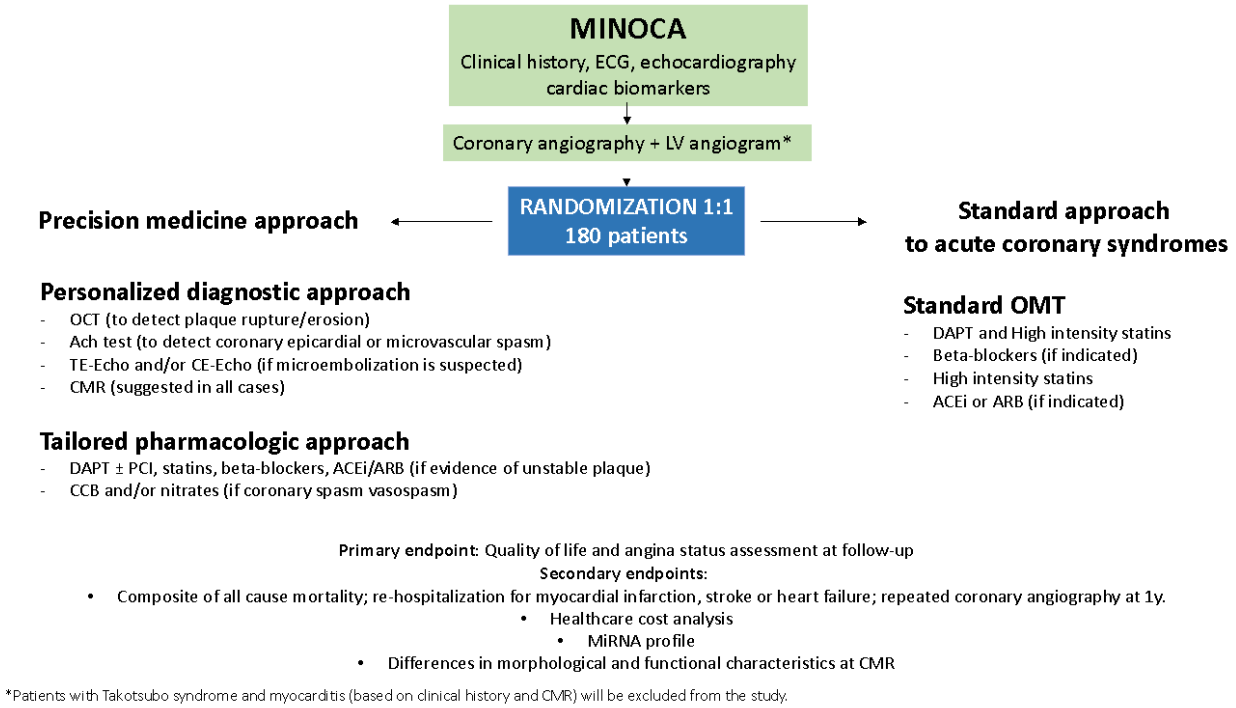
* To reduce the risk of detection and performance bias, a team of 2 cardiologists blinded to group allocation and belonging to an external cardiology unit will submit and collate the questionnaires from study participants.

Secondary endpoints:

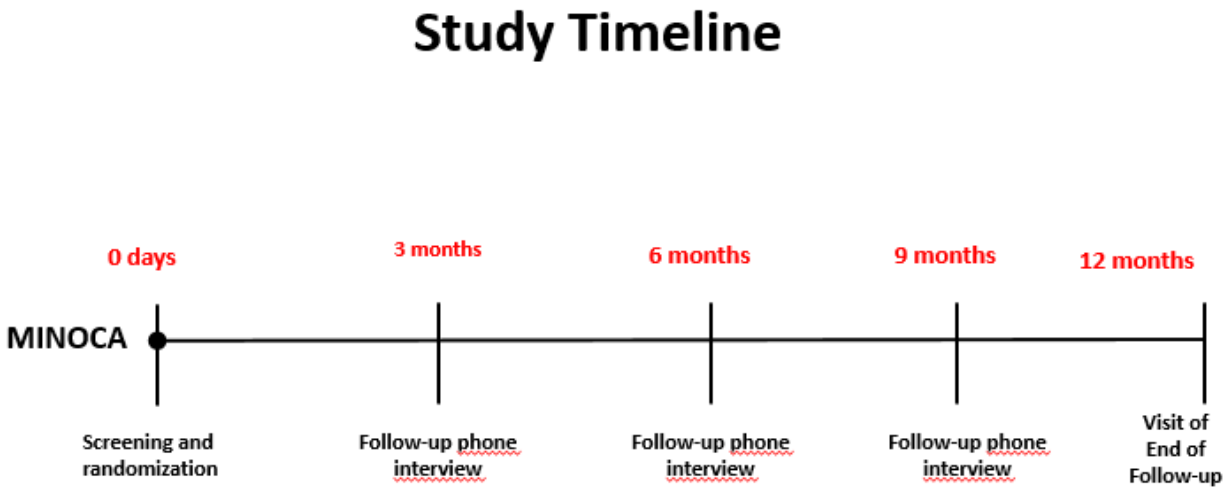
- Rates of major adverse cardiovascular events (MACE; composite of all-cause mortality; re-hospitalization for myocardial infarction, stroke or heart failure; repeated coronary angiography) at 1-year follow-up in MINOCA patients.
- Healthcare primary and secondary related-costs (including costs for tests, procedures and outpatient visits or medicines) and socioeconomic burden of MINOCA patients.
- Ability of different circulating biomarkers (ET-1, NPY, CRP, sCD40L and miRNA [miR-16, miR-26a, miR-145, miR-222, miR-155-5p, miR-483-5p and miR-451]) as diagnostic biomarker and stratification tool for specific causes of MINOCA.
- Ability of CMR in evaluating different mechanisms of MINOCA and their prognostic value.

13. Study Flow Chart

This is a multicenter Italian randomized clinical study in which patients with MINOCA will be enrolled after a careful check of the inclusion and exclusion criteria. The flow chart of the study is resumed in the following **Figure** and schematized in the next sections:



Study timeline



T 0 (index hospitalization for MINOCA):

During the index hospitalization the study investigators, after a careful evaluation of the inclusion and exclusion criteria of the patients, will collect:

- Anagraphic data
- Personal information: weight, height, BMI
- Vital parameters (blood pressure, heart rate, oxygen saturation)
- Therapy at admission and at discharge (concomitant medications included)
- Cardiovascular risk factors: family history of early coronary artery disease (first degree relative with a history of myocardial infarction <60 years), diabetes mellitus [fasting blood glucose >126 mg/dL or treated diabetes mellitus (intake of a diabetic diet or oral hypoglycaemic agents), according to the American Diabetes Association (ADA) criteria], hypercholesterolemia (total cholesterol >200 mg/dL or treated hypercholesterolemia), smoking (current), and hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or treated hypertension).

- Relevant comorbidities: anaemia, chronic kidney disease, atrial fibrillation, history of stroke/TIA, peripheral vascular disease
- ECG features (ST segment elevation/depression or T wave inversions, atrioventricular blocks, arrhythmias)
- Trans-thoracic color-Doppler echocardiography (chamber volumes, systolic and diastolic function)
- Data on coronary angiography and ventriculography (presence/absence of non-obstructive CAD, micro vs epicardial coronary spasm, "hazy" angiographic images, TIMI flow, LVEDP)
- Laboratory data (creatinine, full blood count, Troponin I peak, CK-MB peak, NT-proBNP, CRP)

For patients randomized to *"precision medicine approach"*:

- OCT (if coronary plaque rupture/erosion is suspected) at the time of coronary angiography in the cath-lab.
- Acetylcholine provocative test (to assess the presence of coronary vasospasm) at the time of coronary angiography in the cath-lab.
- TE-Echo and/or CE-Echo (if distal/microvascular embolization is suspected) during the hospitalization at the echo laboratory
- CMR with contrast media: anatomical and morphological data
- Blood sampling for circulating biomarkers and miRNA expression profile at the time or within the 12 hours from coronary angiography. Blood sampling will be processed and analysed in the research laboratory of the Department of Cardiovascular Science. Biological aliquots will be preserved at XBiogem Biobank at Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome.

For patients randomized to *Routine diagnostic work-up:*

- Coronary angiography and ventriculography
- Transthoracic echocardiography in all patients during the index hospitalization
- CMR with contrast media only if clinically indicated (i.e. to exclude myocarditis or takotsubo syndrome)

T1_ Follow-up phone interview at 3 months (90 +/- 5 gg)

- Collection of MACE at follow-up
- Collection of ongoing medical therapy
- Registration of adverse events

T2_ Follow-up phone interview at 6 months (180 +/- 5 gg)

- Collection of MACE at follow-up
- Collection of ongoing medical therapy
- Registration of adverse events

T3_ Follow-up phone interview at 9 months (270 +/- 5 gg)

- Collection of MACE at follow-up
- Collection of ongoing medical therapy
- Registration of adverse events

T4_ Follow-up clinical visit at 12 months (365 +/- 5 gg)

- Personal information: weight, height, BMI
- Collection of ongoing medical therapy
- Compilation of SAQ score
- Collection of MACE at follow-up
- Registration of adverse events

Study Assessment	**Screening and randomization**	**Follow-up phone interview_ T1 (3 months)**	**Follow-up phone interview_ T2 (6 months)**	**Follow-up phone interview_ T3 (9 months)**	**Visit of End of Follow-up (12 months)**
Days	0	90 +/- 5 gg	180+/- 5 gg	270+/- 5 gg	365+/- 5 gg
Weeks	**0**	**12**	**24**	**36**	**52**

Informed consent	x				
Inclusion criteria	x				
Anagraphic data	x				
Personal information (weight, height,BMI)	x				x
Clinical history	x				
Clinical examination	x				
Vital signs	x				
Ongoing medical therapy (concomitant medications included)	x	x	x	x	x
Patients assigned to **"Standard approach"**	x				
ECG features	x				
Transthoracic Echocardiography	x				
Coronary angiography and ventriculography	x				
CMR with contrast media (if clinically indicated)	x				
Laboratory data (creatinine, full blood count, Troponin I peak, CK-MB peak, NTproBNP, CRP)	x				
Patients assigned to **"Precision-medicine approach"**					
OCT (if coronary plaque rupture/erosion is suspected)	x				
	x				

Acetylcholine provocative test (to assess the presence of coronary vasospasm)					
TE-Echo and/or CE-Echo (if distal/microvascular embolization is suspected)	**x**				
CMR with contrast media (if clinically indicated)	**x**				
Blood sampling for circulating biomarkers and miRNA expression profile	**x**				
Collection of MACE	**x**	**x**	**x**	**x**	**x**
Adverse events	**x**	**x**	**x**	**x**	**x**
SAQ score					**x**

SAQ score:

1. The following is a list of activities that people often do during the week. Although for some people with several medical problems it is difficult to determine what it is that limits them, please go over the activities listed below and indicate how much limitation you have had **due to chest pain, chest tightness, or angina over the past 4 weeks.**

Place an x in one box on each line.

Activity	Severely Limited	Moderately Limited	Somewhat Limited	A Little Limited	Not Limited	Limited, or did not do for other reasons
Dressing yourself	❑	❑	❑	❑	❑	❑
Walking indoors on level ground	❑	❑	❑	❑	❑	❑
Showering	❑	❑	❑	❑	❑	❑
Climbing a hill or a flight of stairs without stopping	❑	❑	❑	❑	❑	❑
Gardening, vacuuming, or carrying groceries	❑	❑	❑	❑	❑	❑
Walking more than a block at a brisk pace	❑	❑	❑	❑	❑	❑
Running or jogging	❑	❑	❑	❑	❑	❑
Lifting or moving heavy objects (e.g. furniture, children)	❑	❑	❑	❑	❑	❑
Participating in strenuous sports (e.g. swimming, tennis)	❑	❑	❑	❑	❑	❑

2. Compared with 4 weeks ago, how often do you have **chest pain, chest tightness, or angina** when doing your **most strenuous** level of activity?
I have had **chest pain, chest tightness,** or **angina**...

Much more often	**Slightly more** often	About **the same**	**Slightly less** often	**Much less** often
❑	❑	❑	❑	❑

3. Over the past 4 weeks, on average, how many times have you had **chest pain, chest tightness, or angina?**
I get **chest pain, chest tightness,** or **angina**...

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks
❑	❑	❑	❑	❑	❑

4. Over the past 4 weeks, on average, how many times have you had to take nitros (nitroglycerin tablets) for your **chest pain, chest tightness,** or **angina?**
I take nitros...

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks
❑	❑	❑	❑	❑	❑

5. How bothersome is it for you to take your pills for **chest pain, chest tightness or angina** as prescribed?

Very bothersome	**Moderately** bothersome	**Somewhat** bothersome	**A little** bothersome	**Not** bothersome at all	My doctor has **not prescribed** pills
☐	☐	☐	☐	☐	☐

6. How satisfied are you that everything possible is being done to treat your **chest pain, chest tightness, or angina**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Highly satisfied
☐	☐	☐	☐	☐

7. How satisfied are you with the explanations your doctor has given you about your **chest pain, chest tightness, or angina**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Highly satisfied
☐	☐	☐	☐	☐

8. Overall, how satisfied are you with the current treatment of your **chest pain, chest tightness, or angina**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Highly satisfied
☐	☐	☐	☐	☐

9. Over the <u>past 4 weeks</u>, how much has your **chest pain, chest tightness, or angina** interfered with your enjoyment of life?

It has **severely** limited my enjoyment of life	It has **moderately** limited my enjoyment of life	It has **slightly** limited my enjoyment of life	It has **barely** limited my enjoyment of life	It has **not** limited my enjoyment of life
☐	☐	☐	☐	☐

10. If you had to spend the rest of your life with your **chest pain, chest tightness, or angina** the way it is right now, how would you feel about this?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Highly satisfied
❑	❑	❑	❑	❑

11. How often do you worry that you may have a heart attack or die suddenly?

I can't stop worrying about it	**I often** think or worry about it	**I occasionally** worry about it	**I rarely** think or worry about it	**I never** think or worry about it
❑	❑	❑	❑	❑

Definition of the MACE

MACE analysis includes:

- All cause death:
  - Cardiac death (in which a direct cause attributable to cardiac disease is present);
  - Sudden cardiac death (in which cardiac death occurred out of the hospital and suddenly; or in the hospital due to ventricular arrhythmias unrelated to other concomitant cardiac conditions);
  - Non-cardiac death (in which the event of death is considered not to be a direct consequence of cardiac disease);
- Hospitalization in which the main diagnosis is myocardial infarction, stroke or heart failure;
- Repeated coronary angiography.

The analysis will consider time to first event and time to each event.

Safety parameters will include data deriving from history and physical examination performed at each visit, laboratory data and results of functional and imaging tests. To enhance detection of adverse events between visits, all patients will be encouraged to contact the research team at any time with concerns or any perceived changes in their healthcare status.

14. Sample size calculation: Sample size calculation for the primary endpoint: to detect a mean group difference of change in SAQ score of 9 U, we calculated that a sample size of 70 patients per group (140 patients total) gave 80% power to detect a between-group difference in SAQ. This

calculation assumed a 2-tailed 5% significance level. This projected calculation assumed an SD of 19 U and was consistent with previous studies (see landmark CorMicA trial). However, we extended the sample size at 180 patients to avoid any reduction of statistical power due to patients lost at follow-up or due to poor compliance to medical therapy.

15. Timeline: At 36 months all patients will be enrolled and will have completed the follow-up. The study will be considered ended after the date of last patient last visit (LPLV). Blood samples will be analysed for assessment of circulating biomarkers and miRNA to detect associations with specific mechanisms underlying MINOCA, for stratification of prognosis and to evaluate response to medical therapy. Furthermore, CMR sequences will be analysed to detect functional and structural characteristics associated with specific mechanisms underlying MINOCA and with for stratification of prognosis.

METHODS: INTERVENTION ALLOCATION

16-17 Randomization

Trained staff in the catheter laboratory will use a web-based randomization tool, available 24h/24h at any of the enrolling sites, to immediately randomize the patient after the index coronary angiography revealing non obstructive CAD, to "precision medicine approach" versus "standard approach" (open label approach: both patients and physicians will know the allocated treatment arm).

METHODS: DATA COLLECTION, MANAGEMENT AND ANALYSIS

18. Data collection: Data collection will be carried out through an electronic CRF (eCRF). The investigator or an authorized member of the investigation team must sign all completed eCRFs with a digital signature (a password will be provided by the data management center at the start of the study). The management of clinical data will be carried out in accordance with the data cleaning procedures, both for the data recorded in the eCRFs and from other sources. Appropriate computerized software will be used to verify the accuracy of the database. Investigators will be asked for incomplete, inconsistent or missing data.

Source Documents (SDs): Investigators have to maintain information in the patient's medical record that corroborates the data collected in the eCRF. To meet these demands, the following is a list of information that must be recorded and shown to monitors and regulatory inspectors as needed: Clinical history / physical condition of the participant before inclusion in the study with sufficient detail to verify the inclusion and exclusion criteria; clinical notes dated and signed on the day of entry into the study, protocol number, clinical site, number assigned to the patient and a declaration certifying the signature of the informed consent; record of abnormal laboratory tests; reported adverse events and their resolution; including supporting documents such as discharge sheet, catheterization reports, ECG, echocardiography and CMR reports; laboratory results; condition of the patient upon completion or withdrawal from the study.

Case Report Form: All requested data will be carefully recorded by authorized personnel documented in the Authorized signature log in the eCRF.

19. Confidentiality: All data and information collected during this study concerning the participant must adhere to the privacy protection standards, based on the application of the Italian laws on the

confidentiality of the participating subjects and the European General Data Protection Regulation (GDPR). All data used for the analysis and the study summary will be anonymous and without specific references to the names of participants. Access to the subjects' personal files will be limited to authorized personnel, investigators and research staff. Authorized personnel from regulatory agencies have the right to inspect and copy all records relevant to this study, but every effort must be made to remove the subjects' personal data.

Record Storing: All information in the eCRF, study records, reports and SDs that support the eCRF must be kept in the files of the investigator responsible in accordance with national requirements, and will be stored further in accordance with national and international guidelines as described in the Investigator Site Agreement. This documentation must be accessible if required by international regulatory authorities. The documentation and data collected must be kept for at least 15 years after the completion or suspension of the trial itself.

20. Statistical analysis

The study population will comprise participants who had provided informed consent. There will be no interim analyses, and the trial enrolment will be considered complete after the pre-specified recruitment target was met. Data will be reported as mean ± SD, median (25th, 75th percentile), or frequency and percentage. Continuous outcome measures recorded at baseline and 6 months will be compared between randomized groups using a mixed effects linear regression model, including a random effect for patients, and fixed effects for time point (baseline or follow-up), randomized group, and their interaction. The baseline-adjusted intervention effect will be estimated as the interaction term from this model. Categorical outcomes will be compared between randomized groups using Fisher exact tests with additional calculation of relative risk estimation of effect size. We will perform 2-tailed analysis and considered a p value ≤0.05 to be significant. Statistical analyses will be performed using SPSS program.

METHODS: MONITORING

21.a Data Monitoring

By monitoring we mean the act of supervising the progress of a clinical trial, and ensuring that it is conducted and reported in accordance with the protocol, the Standard Operating Procedures (SOPs), the Good Clinical Practice (GCP), the Good Manufacturing Practice (GMP) and applicable regulations. The purpose is to verify that:

- The rights and health of human subjects are respected
- The data reported is accurate, complete and verifiable on source documents
- The conduct of the trial complies with the approved protocol / amendments / GCP / GMP / and requests from regulatory authorities
- The quality of the trial is verified over time

Monitoring will be carried out by the respective CTUs (Clinical Trial Units) at the sites or by the CRO delegated for this purpose.

A monitoring plan and SOPs describing in detail the documents and data to be monitored will be developed to ensure homogeneous monitoring.

An initiation visit before the start of the trial, visits during and at the end of the trial (close-out visit) are provided for each center. Regular telephone conferences between the CTUs / CROs will ensure the harmonization of procedures.

All data and source documents will be made accessible to the monitors and any questions posed during monitoring can be answered by any participant.

Data Monitoring Committee (DMC)

The DMC will consist of an independent group of 3 experts consisting of a clinical cardiologist expert in cardiovascular emergencies, an interventional cardiologist, and a clinical cardiologist expert in biostatistics. The DMC has experience in managing acute patients undergoing coronary revascularization and conducting a clinical trial. The DMC is responsible for monitoring the safety of participants and the conduct of the PROMISE trial. The DMC provides recommendations on the advisability of continuing the clinical trial or of adopting protocol changes necessary to maintain the safety of enrolled patients. The composition of the DMC will be defined before the start of the clinical trial. The initial DMC meeting will occur at the start of the trial and will convene once annually, to

examine the accumulated safety and enrollment data, review study progress, and discuss all the factors that might impact continuation of the study as designed.

21. It is the responsibility of the PI to report any changes in the research activity and unanticipated problems concerning the risk to human beings; including planned or untimely conclusion of the study and final report. Premature interruption of the study must be reported within 15 days. The regular term of the study will be communicated to AIFA (OsSC) and the CEC within 90 days. The final study report will be reported within 1 year of the end of the study.

22. Adverse events

According to Article 2 of Directive 2001/20/EC "adverse event" is defined as follows:

'Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment'.

"An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product".

Adverse reactions

An 'adverse reaction' is defined according to Article 2(n) of Directive 2001/20/EC as follows:

"All untoward and unintended responses to an investigational medicinal product related to any dose administered".

The definition implies a reasonable possibility of a causal relationship between the event and the IMP.

Serious Adverse Events/Reactions (SAEs/SARs)

A 'serious adverse event/reactions' is defined in Article 2 of Directive 2001/20/EC as follows:

"Any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

These characteristics/consequences have to be considered at the time of the event. For example, regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe".

Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition. Medical and scientific judgement should be exercised in deciding whether an event is 'serious' in accordance with these criteria.

Reports of serious adverse events (SAEs) must be immediately notified to the PI of the Coordinating Center regardless of the attribution by the Investigator of a causal relationship with the product under study.

The causal relationship with the product under study will be defined as follows:

- related: after careful evaluation, a safe relationship emerges with the use of the product under study,
- not related: due to causes not related to the product under study.

Unexpected adverse reactions

An unexpected adverse reaction is defined by the Article 2(p) of Directive 2001/20/EC as follows:

"An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product)".

23. Management and reporting of Serious Adverse Events/Reactions

Any adverse event will be assessed, for

- seriousness, for serious adverse event and serious adverse reaction qualification,

• relatedness with the study drug (related/unrelated), for suspected adverse reaction, qualification

• expectedness, referring to safety reference document, and as per protocol, for suspected unexpected adverse drug reaction - SUSAR identification.

In accordance with Article 16 of Directive 2001/20/EC the investigator is responsible for reporting to PI of the Coordinating Centre all serious adverse events in relation to subjects treated by him in the clinical trial as it reads as follows:

“The investigator shall report all serious adverse events immediately to the sponsor except for those that the protocol or investigator’s brochure identifies as not requiring immediate reporting.”.

Moreover, any related or possibly related or unrelated Serious Adverse Events occurring during the clinical study should be immediately reported to the PI of the study coordinating center within a 24 hours following knowledge of the serious adverse event. Immediate reporting should allow to take the appropriate measures to address potential new risks in a clinical trial. The Investigator will always provide an assessment of causality between the experimental drug/event. It is mandatory for the Principal Investigator (and/or designees) to notify fatal events to the applicable EC.

The communication must be sent via email using the Serious Adverse Events form from the investigators involved in the study.

The form must be sent via email to the following recipient:

• Attention of Dr. Rocco Antonio Montone: email: promise.trial@policlinicogemelli.it

It will be the responsibility of the PI of the coordinating center of the study to follow up on the report by completing and sending the following forms to the Ethics Committees:

- INITIAL SAE REPORT from SAE occurred during the study to the Ethics Committees involved (included in the Investigator's Study File);
- Follow-up REPORT on additional information from the Center to the PI of the coordinating center and from this to the Ethics Committees involved (included in the Investigator's Study File);

- FINAL REPORT on the final and conclusive information to the Ministry from the Center to the PI of the Health Coordinating Center (AC) and to the Ethics Committees involved (included in the Investigator's Study File).

Serious unexpected adverse drug reactions (SUSAR) will be reported to the Competent Authorities, via Eudravigilance and the Ethical Committee within 7 days for fatal and life-threating SUSAR, 15 days for other SUSARs.

All serious adverse events, serious adverse reactions and SUSARs will be presented as listing and a Developmental Safety Annual Report – DSUR will be issued annually from the study approval lock date to AIFA (Agenzia Italiana del Farmaco) and to the applicable Ethics Committees.

RISK AND BENEFIT OF THE STUDY PROTOCOL

The study involves risks of possible adverse events related to the pharmacological therapies administrated and the interventional procedure performed, which, however, are addressed in an expert and high-volume center (e.g. risks related to coronary angiography procedure, OCT or acetylcholine provocative test for patients randomized to “precision-medicine approach ”,CMR, transesophageal echocardiography or transthoracic echocardiography with contrast medium, which however do not expose to ionizing radiation, venous blood draw which could lead to discomfort, pain or small bruises at the puncture site). However, with this prospectively designed trial, we have the opportunity to test the prognostic value of a targeted therapeutic approach based on the identification of the underlying cause. Of importance, the results deriving from our trial may open the way for a new pathophysiology-driven approach with cause-target therapies personalized for the mechanism of MINOCA. Therefore, we retain that the benefits of a precision-medicine approach for the treatment of MINOCA will outweigh all the possible risks associated with this clinical study”.

ETHICAL CONSIDERATIONS AND RESULTS SHARING

The study will be conducted in 3centers in Italy. The authorization by AIFA (OsSC), competent authority on the matter, the single opinion expressed by the coordinating center and the opinions expressed by the respective ethics committees of the clinical centers involved regarding the conduct

of the study will be transmitted, in writing and complete, in all their parts, to the Investigator before the start of the study.

24. Research Ethical Approval

Competent Ethical Committee (CEC): The investigator/ PI responsible for each participating center ensures that the institutional ethics committee has provided a favorable opinion. No changes to the protocol will be made without the prior approval of the Investigator, authorization from AIFA (OsSC) and from the Ethics Committee of the coordinating center and subsequently by the other CECs of the centers involved, except when it is necessary to prevent apparently immediate risk to study participants. In this case, to prevent any serious risk for the patient, the modification to the protocol can be implemented immediately but at the same time submitted to AIFA (OsSC) and to the coordinating EC and to the other ECs involved. Premature interruption of the study must be reported within 15 days. The regular term of the study will be communicated to AIFA (OsSC) and the CEC within 90 days. The final study report will be reported within 1 year of the end of the study.

Competent authorities*:* The approval of the Italian Medicines Agency (AIFA-OsSC) will be obtained before the start of the clinical trial. Severe and unexpected adverse reactions (SAEs), related to the use of the study drugs, are subject to rapid reporting. They will be communicated to the competent authorities by the PI of the clinical site to the PI of the coordinating Clinical Center from the study (s) to the Minister of Health (CA) and to all CEs, where necessary. All other events will be reported in the annual safety report. The description and seriousness of the events, anticipated or not, the causal association and the dosage will be included in the report. Reactions to fatal or life-threatening drugs will be reported as soon as possible, but no later than 7 calendar days. All the others, however, no later than 15 calendar days. The regular term of the study will be reported to the authorities within 90 days and the final report submitted within 1 year of the end of the study.

Ethical conduct of the study: The study will be conducted in accordance with the protocol and principles set out in the latest available version of the Declaration of Helsinki, in the latest version available guidelines of Good Clinical practice (GCP) issued by the ICH, in the latest updates of national laws / regulations and respecting the dictates of the Italian competent authority. The CEC and regulatory authorities will receive periodic and annual safety reports and will be informed about the interruption / suspension of the study in accordance with local requirements.

25. Amendments to the Protocol

The investigator (Dr. Montone) is authorized to make amendments to the protocol. The PIs of each site are allowed to make suggestions for any amendments. Important changes to the protocol (eg change of eligibility criteria, outcomes, statistical analysis) will be communicated within 5 days to the parties involved (investigators, competent authorities (AIFA-OsSC) and CEC, study participants, online registers, regulatory authorities). Substantial amendments will only be implemented after approval by the CEC and the competent authority. In emergency conditions, deviations from the protocol to protect the rights, safety and well-being of participants may be made without the prior approval of the sponsor or the CEC / CEA. Such deviations must be documented and promptly reported to the sponsor, AIFA-OsSC and the CEA. All non-substantial amendments will be communicated to AIFA-OsSC as soon as possible and to the CEC in the context of the Development Safety Update Report (DSUR).

26. Patient Information and Informed Consent and Consent to the Processing of Personal Data

The PIs of each clinical site will explain to patients the nature of the study, its rationale, the procedures involved, the duration, the potential risks and benefits, and any possible inconvenience both orally and by means of a written form (Patient Information: Each patient will be informed about the voluntary participation in the study and the possibility, at any time, to withdraw from the study without incurring any deterioration in the quality of medical assistance and treatments. The patient's data will be anonymized after the withdrawal of informed consent and the patient will be informed about any alternative treatments. Patients should be informed that other authorized individuals, in addition to their doctor, may review their personal data and will issue a consent to the processing of personal data in this regard. All study participants will be provided with sufficient information to make an informed decision about participating in the study. They will be given the opportunity to discuss treatment options and participation in the study with relatives or other trusted persons. The patient information sheet, the consent form and the consent form for the processing of personal data will be submitted to the respective CEC and the competent authorities (OsSC) for review and approval. The participant's formal consent, accompanied by the appropriate approved forms, must be obtained before submitting the participant to any procedure. Participants should read and consider the information received before dating and signing the consent form and attached forms, a copy of which will be provided to them. The consent form and the attached forms will also be signed by the investigator (or his delegate) and will be kept in the official documents of the study.

27. Confidentiality and Data Protection

Direct access to the SD will be guaranteed for the purposes of monitoring, audits and inspections.

The investigator supports and abides by the principle of the participant's right to privacy and compliance with privacy regulations. In particular, the anonymity of the participants will be guaranteed if data is presented at scientific meetings or if they are published in scientific journals.

The medical information obtained as a result of the study is to be considered confidential and its disclosure to third parties is prohibited. Confidentiality will be further guaranteed by the use of identification codes of the subjects to be matched to processing data in digital files.

For verification purposes, relevant agencies (AIFA-OsSC), or CECs may request access to parts of the clinical records relevant to the study, including the participants' medical history.

28. Conflicts of Interest

Investigators participating in the study must ensure that they have no agreement or no economic interest, direct or indirect, with the manufacturers of the study drugs.

29. Audits and inspections

Responsible ethics committees, sponsor and authorities have the right to inspect the practice and / or manufacturing sites at any time before, during and after clinical conduct. There will be no further audits beyond monitoring and inspections. The study documentation and Source Data (SD) will be accessible to auditors / inspectors (including CECs and CAs) and questions will be answered during the inspection. All parties involved must treat participant data as strictly confidential.

Insurance

The promoter will be responsible for providing insurance coverage for the study for all the enrolling sites.

31. Publication Policy

The steering committee and investigators undertake to publish and disseminate the study results. The PROMISE study is a multicenter, randomized study. All public submissions, manuscript generation and submission will be led by the PI, who will create and coordinate a publication committee. This study represents a shared effort between investigators and collaborators and, as such, the parties agree that any party's recommendations regarding the manuscript or text should be taken into account in preparing the final scientific paper for submission or publication. The final database will be kept at the University of Sacred Heart, Rome, which will not release data or material relevant to the study without the permission of the PI. All PIs will be on the list of co-authors on all abstracts

and publications. Therefore, their adherence to the publication is required. Publication and / or submissions based on single clinical site data are not permitted until the results of the multicenter study are published. All data from individual sites will need to be generated from the central database - local databases are not allowed. All proposals for publications and presentations deriving from or related to the study must be submitted to the Steering Committee for review and approval prior to submission for presentation or publication. The Steering Committee will receive the material of each submission / publication proposal prior to submission / submission, in time to be reviewed by all parties.

32. Informed consensus

See the files attached.

All patients must date and sign informed consent first to enter the studio and before any activity related to experimentation.

In case of amendments that could directly affect the continued participation in the trial by the person, further consent must be obtained from the same.

33. Management of biological materials

Blood sample collection: 42 mL venous blood samples will be collected at the time of coronary angiography or within 12 hours from coronary angiography. Biochemical profile for evaluating the expression of Endothelin-1 (ET-1) and Neuropeptide Y (NPY), C-Reactive Protein (CRP), soluble CD40 ligand (sCD40L) will performed on plasma and serum samples at baseline with ELISA kit.
Peripheral blood mononuclear cells (PBMC) will be isolated from EDTA blood by density gradient centrifugation. Serum and PBMC expression levels for deregulated miRNAs will be performed on the same samples to test circulating levels of miR-16, miR-26a, miR-145, miR-222, miR-155-5p, miR-483-5p and miR-451a with Serum microRNA microarray commercial kit. Coded plasma, serum and PBMC samples will be stored at -80°C at FPG XBiogem Biobanc.
All experiments and analysis will be performed at the "Cellular and molecular cardiology laboratory" of the Department of Cardiovascular Science. Coded plasma, serum and PBMC sample from patients enrolled in the other centers (Centro Cardiologico Monzino IRCCS and IRCCS Policlinico San

Donato) will be collected with the same protocol for procedure standardization and will be shipped to our Unit.

34. Ethical considerations:

The trial will be conducted in accordance with the protocol designed for ensure adherence to Good Clinical Practice as indicated in the attachments:

ICH Harmonized Tripartite Guidelines for Good Clinical Practice, 1996. Note for Guidance on Good Clinical Practice CPMP / ICH / 135/95

EU Directive 2001/20 / EC, 2005/28 / EC

Declaration of Helsinki (1964, and its amendments and clarifications).

The PI, or his delegate, accepts, by signing the protocol, to respect as reported.

35. References

1. Tamis-Holland JE, Jneid H, Reynolds HR, et al. Contemporary Diagnosis and Management of Patients With Myocardial Infarction in the Absence of Obstructive Coronary Artery Disease: A Scientific Statement From the American Heart Association. Circulation. 2019 Mar 27:CIR0000000000000670ò
2. Niccoli G, Scalone G, Crea F. Acute myocardial infarction with no obstructive coronary atherosclerosis: mechanisms and management. Eur Heart J. 2015;36:475-81.
3. Del Buono MG, Montone RA, Iannaccone G, Meucci MC, Rinaldi R, D'Amario D, Niccoli G. Diagnostic work-up and therapeutic implications in MINOCA: need for a personalized approach. Future Cardiol. 2020 Jul 6. doi: 10.2217/fca-2020-0052. Epub ahead of print. PMID: 32628045.
4. Safdar B, Spatz ES, Dreyer RP, et al. Presentation, Clinical Profile, and Prognosis of Young Patients With Myocardial Infarction With Nonobstructive Coronary Arteries (MINOCA): Results From the VIRGO Study. J Am Heart Assoc. 2018;7(13).
5. Grodzinsky A, Arnold SV, Gosch K, et al. Angina Frequency After Acute Myocardial Infarction In Patients Without Obstructive Coronary Artery Disease. Eur Heart J Qual Care Clin Outcomes. 2015;1(2):92-99.

6. Planer D, Mehran R, Ohman EM, White HD, Newman JD, Xu K, Stone GW. Myocardial Infarction Prognosis of Patients With Non–ST-Segment–Elevation Myocardial Infarction and Nonobstructive Coronary Artery Disease. June 2014:1-9.
7. Jespersen L, Abildstrøm SZ, Hvelplund A, et al. Symptoms of angina pectoris increase the probability of disability pension and premature exit from the workforce even in the absence of obstructive coronary artery disease. Eur Heart J. 2013 Nov;34(42):3294-303.
8. Montone RA, Niccoli G, Fracassi F, et al. Patients with acute myocardial infarction and non-obstructive coronary arteries: safety and prognostic relevance of invasive coronary provocative tests. Eur Heart J. 2018;39:91-98.
9. Montone RA, Niccoli G, Russo M, Giaccari M, Del Buono MG, Meucci MC, Gurguglione F, Vergallo R, D'Amario D, Buffon A, Leone AM, Burzotta F, Aurigemma C, Trani C, Liuzzo G, Lanza GA, Crea F. Clinical, angiographic and echocardiographic correlates of epicardial and microvascular spasm in patients with myocardial ischaemia and non-obstructive coronary arteries. Clin Res Cardiol. 2020 Apr;109(4):435-443. doi: 10.1007/s00392-019-01523-w. Epub 2019 Jul 3. PMID: 31270616.
10. Crea F, Niccoli G. Myocardial Infarction With Nonobstructive Coronary Atherosclerosis: The Need for Precision Medicine. JACC Cardiovasc Imaging. 2018 Oct 12. pii: S1936-878X(18)30807-6.
11. Pasupathy S, Air T, Dreyer RP, Tavella R, Beltrame JF. Systematic review of patients presenting with suspected myocardial infarction and nonobstructive coronary arteries. Circulation. 2015;131:861-70.
12. Dastidar AG, Baritussio A, De Garate E, Drobni Z, Biglino G, Singhal P, Milano EG, Angelini GD, Dorman S, Strange J, Johnson T, Bucciarelli-Ducci C. Prognostic Role of Cardiac MRI and Conventional Risk Factors in Myocardial Infarction With Nonobstructed Coronary Arteries. JACC Cardiovasc Imaging. 2019. pii: S1936-878X(19)30065-8.
13. Lindahl B, Baron T, Erlinge D, et al. Medical Therapy for Secondary Prevention and Long-Term Outcome in Patients With Myocardial Infarction With Nonobstructive Coronary Artery Disease. *Circulation*. 2017;135(16):1481-1489.
14. Ford TJ, Stanley B, Good R, et al. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. J Am Coll Cardiol. 2018;72(23 Pt A):2841-2855. doi:10.1016/j.jacc.2018.09.006

15. Ford TJ, Stanley B, Sidik N, et al. 1-Year Outcomes of Angina Management Guided by Invasive Coronary Function Testing (CorMicA). JACC Cardiovasc Interv. 2020;13(1):33-45. doi:10.1016/j.jcin.2019.11.001
16. Rosano GMC, Tousoulis D, McFadden E, Clarke J, Davies GJ, Kaski JC. Effects of neuropeptide Y on coronary artery vasomotion in patients with microvascular angina. Int J Cardiol. 2017 Jul 1;238:123-127.
17. Toyo-oka T, Aizawa T, Suzuki N, Hirata Y, Miyauchi T, Shin WS, Yanagisawa M, Masaki T, Sugimoto T.Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. Circulation. 1991 Feb;83(2):476-83.
18. Fong SW, Few LL, See Too WC, Khoo BY, Nik Ibrahim NN, Yahaya SA, Yusof Z, Mohd Ali R, Abdul Rahman AR, Yvonne-Tee GB. Systemic and coronary levels of CRP, MPO, sCD40L and PlGF in patients with coronary artery disease. BMC Res Notes. 2015 Nov 14;8:679.
19. Jaguszewski M, Osipova J, Ghadri JR, Napp LC, Widera C, Franke J, Fijalkowski M, Nowak R, Fijalkowska M, Volkmann I, Katus HA, Wollert KC, Bauersachs J, Erne P, Lüscher TF, Thum T, Templin C. A signature of circulating microRNAs differentiates takotsubo cardiomyopathy from acute myocardial infarction. Eur Heart J. 2014 Apr;35(15):999-1006.
20. Circulating Arterial Microrna Profiles Identify Variant Angina Among Patients Presenting Chest Pain. Circulation. 2017;136:A12288
21. Li S, Lee C, Song J, Lu C, Liu J, Cui Y, Liang H, Cao C, Zhang F, Chen H. Circulating microRNAs as potential biomarkers for coronary plaque rupture. Oncotarget. 2017 Jul 18;8(29):48145-48156.

36. Study Promoter: IRCCS Fondazione Policlinico Universitario A. Gemelli

Principal Investigator Site*:* Fondazione Policlinico Universitario Agostino Gemelli (FPG-IRCCS); Largo Francesco Vito, n. 1 – 00168 – Roma.

Other investigator sites involved in the study:

- IRCCS Centro Cardiologico Monzino:
  Principal Investigator: Dr. Nicola Cosentino *nicola.cosentino@cardiologicomonzino.it*
  Via Carlo Parea, 4 – 20138 Milano (MI)
- IRCCS Policlinico San Donato:
  Principal Investigator: Dr. Mario Bollati *mario.bollati@gmail.com*

Piazza Edmondo Malan, 2 – 20097 San Donato Milanese (MI)