RESEARCH PROTOCOL

# Scientific title

**Re**storing microvascular circulation with **d**iagnostic **u**ltrasound and **c**ontrast ag**e**nt: the REDUCE Trial

# Simplified title

REDUCE Trial

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#  Glossary of abbreviations

|  |  |
| --- | --- |
| Abbreviation | Term |
| AE | adverse event |
| AR | adverse reaction |
| AMI | acute myocardial infarction |
| CMRI | cardiac magnetic resonance imaging |
| CRF | case record form |
| DM | diabetes mellitus |
| DSMB | Data Safety Monitoring Board |
| DUS | diagnostic ultrasound |
| ECG | electrocardiogram |
| ED | Emergency department |
| EF | ejection fraction |
| EFS | event-free survival |
| GLS | global longitudinal strain |
| HREC | Human Research Ethics Committee |
| ICD | implantable cardioverter defibrillator |
| iMR | index of microcirculatory resistance |
| IS | infarct size |
| LVEDV | left ventricular end diastolic volume |
| LVESV | left ventricular end systolic volume |
| LVO | left ventricular opacification |
| MCE | myocardial contrast echocardiography |
| MI | mechanical index |
| MRI | magnetic resonance imaging |
| MVO | microvascular obstruction |
| NBMLHD | Nepean Blue Mountains Local Health District |
| PICF | Patient information and consent form |
| PCI | percutaneous coronary intervention |
| QC | quality control |
| RGO | Research Governance Office |
| SAE | serious adverse event |
| STEMI | ST-segment elevation myocardial infarction |
| TMG | Trial management group |
| TSC | Trial steering committee |
| US | ultrasound |

# Administrative information

## Trial registration

The trial was registered in the Australian New Zealand Clinical Trials Registry on the 11th of August 2020, prior to recruitment commencement (Registration number ACTRN12620000807954)([www.anzctr.org.au](http://www.anzctr.org.au)).

## Sponsor

|  |  |
| --- | --- |
| Trial sponsor | University of Sydney |
| Contact name | Professor Kazuaki Negishi |
| Address | Cardiology Department, Nepean HospitalLevel 5, South Block62 Derby Street, Kingswood, NSW 2747  |

## Expected duration of study

Participants will be recruited between September 2020 and December 2023 across three to five Australian tertiary referral hospitals. Follow-up will be conducted at 1 and 6 months after the PCI procedure. It is estimated that data collection will be completed by June 2023.

## Investigators

*Coordinating principal investigator:*

Professor Kazuaki Negishi

Professor of Medicine, Nepean Clinical School, The University of Sydney

Consultant, Department of Cardiology, Nepean Hospital

*Co-investigators:*

Dr. Faraz Pathan

Head of Cardiac Imaging and Cardiac Research, Department of Cardiology, Nepean Hospital

Staff specialist, Department of Cardiology, Nepean Hospital

Dr. Hisham Hallani

Director, Department of Cardiology, Nepean Hospital

Dr. Tom Ford

Staff Specialist, Interventional Cardiology, Gosford Hospital

Dr. Prajith Jeyaprakash

Cardiologist, PHD candidate, Department of Cardiology, Nepean Hospital

A/Prof David Platts

Senior Staff Specialist, Prince Charles Hospital, Brisbane, Qld

Professor Andrew Boyle

Clinical Dean, Hunter Clinical School, University of Newcastle

Cardiologist, Department of Cardiovascular Medicine, John Hunter Hospital

## Stakeholder involvement

The research protocol was developed in consultation with the technicians and clinicians in the cardiac catheterisation and imaging laboratories of Nepean Hospital, Gosford Hospital, John Hunter Hospital and Launceston General Hospital.

# Introduction and background

## Background

Ischaemic heart disease is the leading cause of morbidity and mortality globally [1, 2]. Primary percutaneous coronary intervention (PCI) to ST-elevation myocardial infarction (STEMI) is the established evidence-based standard of care [3]. There have been, however, no significant improvement in mortality for patients diagnosed with STEMI over time [4].

One of the reasons for this is microvascular obstruction (MVO). Even though the high (> 90%) success rate for primary PCI [5], approximately 60% of patients with successful primary PCI had MVO [6, 7], known independent predictor of adverse prognosis. Thrombus aspiration in addition to primary PCI do not improve the prognosis, nor intracoronary administration of low dose tissue plasminogen activator [8].

Sonothrombolysis is the application of high mechanical index diagnostic ultrasound (US) with an ultrasound contrast agent, also known as myocardial contrast echocardiography (MCE). Pre-clinical animal studies have shown that sonothrombolysis can restore epicardial and microvascular flow in acute STEMI. A recent single centre clinical trial demonstrated that sonothrombolysis, delivered before and after primary PCI, increases patency of the infarct vessels, improve microvascular flow, reduce infarct size, and improve ejection fraction (EF) at 6 months. There are, however, four unanswered key questions in previous studies [9]. They are:

1. Can sonothrombolysis improve myocardial salvage in the setting of STEMI
2. Can sonothrombolysis be feasibly administered before and after primary PCI in a multicentre setting?
3. Is sonothrombolysis cost effective?
4. What is the real-time effect of sonothrombolysis on coronary microcirculation in the setting of STEMI

Thus, the aim of this trial is to compare the efficacy of pre- and post-PCI sonothrombolysis against sham echo in patients with a diagnosis of acute STEMI in Australian tertiary hospitals.

## Risk/benefit assessment

### Known potential risks

Most of the procedures in this trial are part of usual practice and carry acceptable risks in line with best clinical practice for the diagnosis and treatment of acute STEMI.

*Electrocardiogram*

This is a safe procedure. Patients may experience minor discomfort when removing the electrodes from their chest, similar to removing a bandage. Rarely, redness or swelling may be experienced as a result of a reaction to the electrode adhesive.

*Coronary Angiogram and Percutaneous Coronary Intervention*

This imaging and interventional procedure carries some risk, including radiation exposure from the X-rays used. Major complications are rare but potential complications include: bleeding, stroke, renal impairment, acute myocardial injury, injury to the catheterised artery (including dissection), irregular heart rhythms, and allergic reactions to the dyes/medications used during the coronary angiogram.

Complications during or soon after a PCI procedure may happen. These could include: bleeding, bruising at the site of sheath (radial or femoral) insertion, infection and change in heart rhythm.

During PCI, we may assess the index of microcirculatory resistance (iMR) using an additional wire (similar to normal coronary wire with pressure-thermodilution function). The risk of procedure is within the risk of PCI because this will be done as part of routine PCI procedure.

*Echocardiogram*

There are no risks involved in a standard transthoracic echocardiogram. However, a patient may feel some discomfort from the transducer as it is held firmly against the chest to obtain the best images of the heart.

*Magnetic resonance imaging*

MRI machines are semi-enclosed tube-shaped magnets. Some individuals may experience discomfort while lying inside an MRI machine. If contrast dye is used, there is a risk of an allergic reaction to the dye.

*Collection of blood sample*

There may be some soreness or tenderness at the injection site. Although rarely, the site can become infected.

*Sonothrombolysis*

Sonothrombolysis is basically the therapeutic application of a clinical diagnostic test of MCE, which is the application of high mechanical index diagnostic ultrasound with a contrast agent. MCE is a rapid bedside method for assessing myocardial perfusion both at rest and stress. It has been performed safely in at least 185,000 patients, in published literature [10-14], as well as in the acute STEMI clinical population [15-17]. This technique has also been used in areas other than the heart, such as the skeletal muscle [18], skin [19], brain [20], and kidney [21].

The contrast agent to be used for the sonothrombolysis is Definity®, the contrast agent used clinically by the participating hospitals.

Product information for Definity®: Serious cardiopulmonary reactions and acute hypersensitivity reactions, including fatalities, have occurred uncommonly during or following perflutren-containing microsphere administration, typically within 30 minutes of administration. The most common adverse reactions are headache, back/renal pain, flushing, nausea, chest pain, injection site reactions, dizziness [22] (Attachment 1).

### Known potential benefits

Benefits of sonothrombolysis have included:

* Increased patency of the infarct vessels
* Improved microvascular flow
* Reduced infarct size
* Improved EF at 6 months
* Less need for ICD [15, 23].

The underlying mechanisms for these benefits are from nitric oxide production from the endothelium [24-26] and the massive release of adenosine triphosphate from endothelial and erythrocyte sources [24].

### Assessment of potential risks and benefits

We believe that the potential clinical benefit to patients from the procedures included in this study outweigh the risks mentioned above. The use of Definity® as a contrast agent during diagnostic ultrasound is well established [10-14].

# Keywords

ultrasound, ultrasound therapy, acute myocardial infarction, microbubbles, microvascular obstruction

# Trial objectives and outcomes

The objectives and outcomes for this trial are outlined in Table 1. Additional information are described in the following sections of this protocol and the Data collection sheet (Appendix 4).

Table . Objectives and outcomes for this trial

|  |  |
| --- | --- |
| OBJECTIVE | OUTCOME & OUTCOME MEASURE |
| Primary |  |
| The primary objective of this trial is to evaluate the impact of add-on sonothrombolysis to primary PCI on infarct size at Day 4±2 in patients with STEMI for the first time. | Infarct size at Day 4±2 by CMRI after PCI, assessed as volume of late Gadolinium enhancement (expressed as % of total myocardium), and LV Ejection Fraction |
| Secondary |  |
| Efficacy of sonothrombolysis | * Before PCI: 1) Chest pain (Scale 1-10), 2) Rate of ST-segment resolution, and 3) Angiographic Recanalization rate
* In-patient (first 24 hours): % ST segment resolution and area under the CPK curve
* In-patient: CMRI at Day 4±2 days after PCI: Sizes of MVO, Myocardial Salvage Index as well as LVEDV, LVESV, and EF
* At 6±1 months post-PCI, 1) CMRI (LVEDV, LVESV, EF, and IS), 2) Echo (LVEDV, LVESV, EF, and GLS)
* Six-month event-free survival (EFS), defined as the time from the start of treatment to first cardiac event or death as a first event (MACE). Cardiac events include left ventricular remodelling, death, non-fatal myocardial infarction (recurrence), congestive heart failure, ventricular arrhythmias and need for prophylactic defibrillator (primary and secondary).
 |
| Safety endpoints | * Arrhythmias during the sonothrombolysis and index admission
 |
| Exploratory  | * index of microcirculatory resistance (iMR) at the end of primary PCI
 |

# Study design

## Overall design

This is a prospective, randomised controlled trial with three arms. The primary endpoint is the infarct size assessed by cardiac MRI at Day 4±2 days post-PCI. The intervention will take place while the participant is admitted to the catheterisation laboratory for a PCI and will be followed up at 1±0.5 month and 6±1 months after their PCI procedure. Randomisation will be performed with a 1:1 allocation, stratified by participating site and infarct territory (anterior or non-anterior by ECG). Participants, PCI operator, and image assessors will be blinded to their allocated group. Participants in the control arm will receive standard care for patients diagnosed with a STEMI and undergoing PCI.

The trial will be conducted in three to five large, tertiary referral hospitals in Australia. The Cardiology departments, catheterisation and imaging laboratories of Nepean Hospital, Kingswood, New South Wales, Gosford Hospital, Gosford, NSW, John Hunter Hospital, Newcastle, NSW and Prince Charles Hospital, Brisbane, QLD will be involved.

## Justification for dose

The amount of contrast agent will be as per standard instructions for Definity®.

## Trial population

The target population for this trial will include patients who have been diagnosed with their first STEMI who are 30-80 years of age of both sexes and who are about to undergo PCI will be recruited into the trial.

## Eligibility criteria

Participants will be assigned to a randomised trial treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

### Inclusion criteria

Each patient must meet all of the following criteria to be enrolled in this trial:

* Age between 30 to 80 years.
* Chest Pain with ST segment elevation >0.1 mV in two contiguous leads
* Eligible for emergent PCI/antithrombotic/antiplatelet therapy.
* Adequate apical and/or parasternal images by echocardiography.
* No contraindications or hypersensitivities to ultrasound contrast agents.

### Exclusion criteria

Patients meeting any of the following criteria will be excluded from the trial:

* Unable to provide valid consent to participate in the trial
* Chest pain lasting >6 hours
* Cardiogenic shock
* Fibrinolytic therapy prior to arrival in the emergency department
* Life expectancy of less than six months from any other co-morbidity or terminally ill
* Prior ST-segment elevation myocardial infarction (STEMI)
* Known or suspected hypersensitivity to perflutren, the contrast agent used for the study
* Known cardiomyopathy
* Known severe valvular heart disease
* Known bleeding diathesis or contraindication to glycoprotein 2b/3a inhibitors, anticoagulants, or aspirin
* Known large right to left intra-cardiac shunts
* Pregnancy

## Lifestyle considerations

Not applicable.

## Screen failures

Screen failures are defined as participants who consent to participate in the trial but who are found, during the screening procedures, to be ineligible to continue in the trial. They therefore are not randomised.

## Recruitment and identification of potential participants

Potential participants will be recruited from the cardiac catheterisation laboratories/ Emergency Departments of three to five Australian tertiary, referral hospitals. Laboratories are available 24 hours a day, seven days of the week. Study recruitment will be conducted across the opening hours at Nepean Hospital. Other enrolling sites (Gosford, John Hunter and Prince Charles Hospitals) with recruit patients from 7am to 7pm on weekdays

Patients aged 30-80 years of either sex, admitted to the catheterisation laboratory / Emergency Department diagnosed with a STEMI will be asked to participate. A total of 150 participants will be included in the study, with 75 participants in each arm.

It is anticipated that each site will have >100 patients admitted with a diagnosis of STEMI per year. Hence, we plan to recruit participants over 12 months.

A STEMI is identified by the emergency response teams as patients with chest pain and ECG evidence (two contiguous leads with >0.1 mV ST elevation or >0.2 ST depression in V2-V4).

In the catheterisation laboratory/ Emergency Department, a Cardiology Advanced Trainee or delegated cardiologist will approach potential patients and invite them to participate in the trial, assess the patient for eligibility and obtain their consent to participate in the study. Figure 1 shows the patient selection process.

Patients that do not meet the inclusion criteria will not be randomised but will serve as a registry for determining angiographic recanalization rates immediately before PCI.

Patients will not receive compensation or incentives for trial participation.

Figure . Patient selection process

Control arm (n=75)

Patients w

/

AMI at

Catheterisation

Laboratory

Patients w

/

STEMI

Non

-

STEMI

excluded

Patients w

/

STEMI

meeting inclusion

criteria randomised

into three arms

Exclusion criteria

**Patient selection**

**:**

Patients that arrive in the catheterisation laboratory will be

selected according to the inclusion and exclusion criteria

.

Patients eligible for the

study will be randomised to three arms

.

AMI

=

acute myocardial infarction

;

STEMI

=

ST

-

segment elevation myocardial infarction

;

PCI

=

percutaneous coronary

intervention

.

Pre and Post PCI Sonothrombolysis (n=75)

## Consent

Prior to performing any trial-specific procedure (including screening procedures to determine eligibility), a valid consent will be obtained for each participant. The consent process will include two stages.

Step 1

The process will be that the investigator or delegated member of the trial team will discuss the trial with the participant and answer any questions that the participant may have.

The investigator will provide the Simplified Participant Information and Consent Form (PICF) to the participant which describes the purpose of the trial, the procedures to be followed, and the risks and benefits of participation.

The investigator will conduct the informed consent discussion and will check that the participant comprehends the information provided.

The participant will be invited to provide a verbal or written consent. Consent will be voluntary and free from coercion.

The investigator who conducted the consent discussion will also sign the informed consent form.

It will be documented in the participant’s record that consent has been provided. When all the inclusion/exclusion criteria have been addressed and the eligibility of the participant confirmed, the participant may be assigned to a trial arm/intervention.

Where practical, a copy of the full consent form will be given to the participant while in the Coronary Care Unit (CCU).

Step 2

As soon as it is practicable for the participant and the cardiologist, a second consent will be obtained from the participant using the full consent form. The participant will be asked to provide a written consent. A copy of the consent form will be given to the participant.

A record of potential participants screened but who were subsequently found to be ineligible for the trial will be kept in paper and electronic form as part of the data collection process, further described in Section 14. The following information will be kept: name, MRN, age, sex, reason/s for ineligibility.

# Intervention

## Treatment arms

Figure 2 (page 16) depicts the path of the three study arms for this study. Patients will be randomised to one of three arms:

1. **Group 1: Pre- and Post-PCI arm**

Participants in this group will undergo sonothrombolysis before and after their PCI procedure.

This is an interventional DUS therapeutic group that will receive frequent image-guided diagnostic high MI (1.8 MHz; 1.1 – 1.3 MI; <5-µs pulse duration) impulses applied to the myocardial contrast-enhanced areas (using Definity®) in the apical 4-, 2-, and 3-chamber views before and after PCI.

1. **Group 2: Control arm**

Participants in this group will undergo a sham echocardiography study before and after their PCI procedure.

Sham echocardiography study

Participants will undergo low MI (<0.2) imaging only to assess regional wall motion before and/or after PCI with Definity® infusion.

## Trial intervention(s)

### Description of trial investigational products

#### Contrast agent

The following information is an excerpt from the publicly available product information for Definity® [22]:

“Definity® is an injectable cardiovascular ultrasound contrast agent comprised of lipid-coated echogenic microbubbles filled with octafluoropropane gas that enhances clinicians’ view of the left ventricle of the heart during an echocardiogram to aid with diagnosis.

This medicinal product is for diagnostic use only. DEFINITY® is indicated for use in patients in contrast-enhanced diagnostic ultrasound imaging to improve the characterization of focal lesions of the liver and kidney. DEFINITY is indicated for use in patients with suboptimal echocardiograms to provide opacification of cardiac chambers, improvement of left ventricular endocardial border delineation and assessment of regional wall at both rest and stress.”

|  |  |
| --- | --- |
| **Active substance** | Perflutren lipid microshere, single use 2mL clear glass vial containing clear liquid |
| **Trade or Generic name** | Definity® |
| **Dosage form** | Injectable suspension |
| **Route of administration** | Intravenous (IV) bolus or infusion |

### Dosage

The commercially-available microbubbles (Definity®) to be utilized for these studies will be manufactured by Lantheus Medical. In each session, one vial (1.5 millilitres) will be mixed with approximately 48.5 millilitres of saline (approximately a 3% infusion) and then infused at the rate of 1~2ml/min (adjusted with image quality).

### Storage, preparation, dispensing and administration of trial drug

As per Definity® product information sheet (Appendix 1) [22]. Definity® will be injected by an intravenous infusion.

### Product accountability

The investigator’s designee will maintain accurate records of the receipt and use of all trial medication, including dates of receipt.

### Measurement of participant compliance

Not applicable.

### Excluded medications and treatments

Not applicable.

### Concomitant therapy

Not applicable

### Discontinuation from trial intervention

A participant may stop the trial intervention but will continue with follow-up procedures and assessments on an intention to treat basis. Possible reasons for discontinuation from trial intervention may include:

* Unexpected adverse reaction to the contrast agent;
* Change in their medical condition.

# Randomisation and blinding

Once consented, the participating sites will determine the randomised treatment using an electronic website that is available seven days a week.

We will use the REDCap Randomisation Module to allocate participants into the study groups. Only research staff delegated to this task will be given rights to randomise a participant. Access to the randomisation page will be through the study’s REDCap page where data will be stored as described in Section 14 Data Management.

Participants will be randomised to two intervention groups, with a 1:1 allocation ratio, stratified by site and infarct territory (anterior or non-anterior by ECG). Participants, PCI operators and those taking the measurements from CMRI/echocardiography will be blinded to the participants’ group allocation. The sonographer/imaging specialist performing sonothrombolysis and/or acquiring the images will not be blinded.

## Breaking of the trial blind

### On trial

The randomisation code for an individual participant may only be unblinded in emergency situations, where the Investigator decides a participant cannot be adequately treated without knowing the identity of their treatment allocation. To break the randomisation code, the Investigator must contact the randomisation facility/personnel.

### On completion of the trial

Group allocation codes will only be available once all data collected have been entered into the trial database for every participant and the database has been finalised, except in the case of an emergency, as detailed above.

# Trial visits and procedures

## Trial timeline

As previously described, participants will be admitted to the catheterisation laboratory where their suitability for the trial will be assessed and their consent sought. They will then be randomised to one of the two arms of the study. The interventions will be conducted during their admission for a STEMI and PCI. Follow up will be conducted at one and six months after their PCI procedure. Please refer to Figure 2 (page 15) for a diagram of the study design.

## Schedule of assessments

Please refer to the data collection sheet for additional information (Appendix 4). Follow up visits to the hospital will be kept to a minimum, where possible. In line with NSW Health’s COVID-19 Guidance on Clinical Trials, telehealth, web-based and other methods will be used during the data collection period and for keeping in contact with trial participants.

Figure . Study design of the trial



## Description of procedures

All randomised patients will immediately receive a 2013 AHA/ACC Guidelines approach [27], including aspirin (300 mg) and clopridogrel (300-600 mg), Prasugel (60 mg), or Ticagrelor (180 mg), and atorvastatin (40 or 80 mg), unless contraindicated, as per usual practice within the catheterisation laboratories involved. This will be followed by primary PCI using heparin ± glycoprotein 2b/3a inhibitors as anti-thrombotic agents using established local protocols. Beta-blockers will be administered to all patients while in hospital, unless contraindicated. All pharmacologic regimens will be recorded on patient CRFs.

Sonothrombolysis

All patients will immediately receive an intravenous infusion of 3% Definity® with continuous monitoring of the risk area by transthoracic very low mechanical index imaging (E.g. Philips iE33 or EPIC; Andover, MA) using the low MI (<0.2) contrast LVO setting.

Intermittent high MI impulses (> 1.0 MI; Frequency 1.8 MHz) will be administered over the microvasculature (apical four, two, and three chamber window) for Group 1 (pre- and post-PCI) (Figure 2). Foreshortened windows may be utilized to improve myocardial visualization for high MI impulses. All views can be foreshortened if necessary to improve high MI impulse delivery to basal segments.

**Group 1 Intervention Arm:**

**Pre-PCI** sonothrombolysis will be at least 5 high MI administrations in each window; minimum of 15 high MI administrations over the treatment period for up to 5-10 minutes depending on time constraints in getting to the catheterisation laboratory) while also monitoring for replenishment within the risk area.

**Post-PCI** sonothrombolysis will be at least 10 high MI administrations in each window; minimum of 30 high MI administrations over the treatment period for up to 20-30 minutes immediately after the PCI procedure, while also monitoring for replenishment within the risk area.

Therefore, **Group 1**, where indicated in the study design, will receive multiple brief 10 frame high mechanical index diagnostic impulses in each of the three apical planes with emergent PCI.

Group 2 **Sham Control arm:**

**This group** will receive emergent PCI alone, and a sham echocardiogram with low mechanical index contrast imaging before (5-10 min duration) and after (20-30 min duration) the PCI procedure. The low mechanical index (MI) contrast imaging setting will be 0.2 or less. Gain settings, while in low MI, will be set at between 60-70% and frame rate will be 20-25 Hertz. This regimen will continue during the initial pharmacologic treatments. Pharmacologic agents used during PCI therapy will include either heparin or glycoprotein 2b/3a inhibitor using established protocols.

Medical therapy

The doses of heparin, aspirin, and Plavix/Prasugel/Ticagrelor will be the same as those used for patients undergoing primary PCI therapy as per usual practice.

In-patient and out-patient protocol

Prior to hospital discharge (4±2 days post-PCI), a gadolinium enhanced magnetic resonance scan (LGE-MRI) will be obtained to quantify infarct size and extent of microvascular obstruction [28]. A standard echocardiography will be obtained before discharge as a part of standard clinical practice. Serial ECG (90 minutes and four hours) and serum CPK measurements (at 6 hours and 24 post STEMI) after admission to calculate % ST segment resolution and area under the CPK curve, respectively.

In-hospital and outpatient major adverse cardiovascular events (MACE) will be defined as death, non-fatal myocardial infarction (recurrence), the occurrence of congestive heart failure, or sustained ventricular arrhythmias using standard guidelines-based definitions [29]. All patients will be required to return for a 1-month clinical follow-up. Cardiac MRI will be performed at 6 months to assess infarct size. Follow up at six months will also include clinical endpoints (MACE) to determine event-free survival. Implantable defibrillators will be placed according to the ACC/AHA/ESC 2013 guidelines for both primary and secondary prevention criteria following myocardial infarction [27]. Congestive heart failure will be defined, using the AMISTAD-II trial criteria [30], as the presence of two of the following criteria: new pulmonary oedema by chest X-ray in the absence of a non-cardiac cause, rales over one-third or greater of the lung fields believed to be due to pulmonary oedema, pulmonary capillary wedge pressure>18 mm Hg combined with a cardiac index <2.4 l/min/m2, dyspnoea with pO2 <80 mm Hg or O2 saturation <90% without pre-existing lung disease, and use of loop diuretics to treat pulmonary congestion. Left ventricular remodelling will be defined as a 20% increase in end diastolic volume at the 6-month follow up biplane contrast enhanced echocardiogram compared to the pre-discharge contrast enhanced echocardiogram. All events will be adjudicated by a Data Safety Monitoring Board (DSMB).

Echocardiography studies, cardiac MRIs, and ECGs will be placed in a restricted network drive with all patient identifiers removed for blinded review by an independent doctor. CRFs will be updated and maintained by each of the coordinators at each participating site. All CRFs will be electronically maintained and accessible only to study coordinators.

## Notes on specific trial visits

### Screening

Screening will take place as soon as the patient is admitted in the catheterisation laboratory/ Emergency Department and will need to be completed thoroughly but as promptly as possible. There is a short amount of time available before the PCI procedure, if deemed appropriate for the particular individual, needs to be initiated. Additional information have been provided in the preceding sections of this protocol.

### Final trial visit

Where applicable, the investigators will ensure that referrals to the appropriate specialists or services are in place for the participant. Participants will be informed that the results of the trial, through publications, will be made available on The University of Sydney website, under Professor Negishi’s academic profile.

### Unscheduled visit

Unscheduled visits will be noted in the participant’s CRF and if the visit relates to a safety event, the HREC will be notified.

## Treatment discontinuation, participant withdrawals and losses to follow up

### Discontinuation of treatment – participant remains in trial for follow-up

Participants who discontinue trial treatment will remain in the trial. The remaining trial procedures should be completed as indicated by the trial protocol. Participants may discontinue trial treatment for the following reasons:

* Participant request to discontinue trial intervention
* Investigator decision to discontinue a participant from the trial intervention if the participant:
	+ Is pregnant
	+ Experiences a serious or intolerable adverse event such that continued trial intervention would not be in the best interest of the participant
	+ Develops, during the course of the trial, symptoms or conditions listed in the exclusion criteria
	+ Requires a medication that is prohibited by the protocol
	+ Requires early discontinuation for any other reason.

The investigator may also withdraw all trial participants from the trial treatment if the trial is terminated.

For the safety of all participants ceasing trial treatment, the protocol-specified safety evaluations should be undertaken to capture new safety events and to assess existing, unresolved safety events.

All scheduled follow-ups of trial participants should also occur following treatment discontinuation, where possible.

A CRF page will capture the date and the reason for the discontinuation. The participant will remain in the trial for scheduled visits for trial assessments (follow-up) per protocol, if possible.

### Withdrawal of consent – participant withdraws from all trial participation

Participants are free to withdraw from the trial at any time upon their request. Withdrawing from the trial will not affect their access to standard treatment or their relationship with the hospital and affiliated health care professionals.

The participant will be asked to complete and sign a withdrawal form (Appendix 5). A CRF page will be used to capture the date of consent withdrawal.

### Losses to follow-up

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the trial site staff.

If a participant fails to return for a required trial visit:

The site will attempt to contact the participant and reschedule the missed visit within two to four weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the trial.

Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant’s last known mailing address or local equivalent methods). These contact attempts will be documented in the participant’s trial file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the trial with a primary reason of lost to follow-up.

### Trial closure

A participant is considered to have completed the trial if he or she has completed all phases of the trial including the last visit or the last scheduled procedure shown in the Schedule of Assessments.

The end of the trial is defined as completion of the last visit or procedure shown in the Schedule of Assessments in the trial at all sites. At this stage, the Investigator will ensure that all HRECs and RGOs, regulatory and funding bodies have been notified.

Temporary halt or early termination of a trial: If the trial is prematurely terminated or suspended, the Investigator will promptly inform trial participants, HREC and RGO, the funding (where applicable) and regulatory bodies, providing the reason(s) for the termination or suspension. Circumstances that may warrant termination or suspension include, but are not limited to:

* Determination of an unexpected, significant, or unacceptable risk to participants, i.e. a significant safety issue
* Insufficient compliance to protocol requirements
* Data that are not sufficiently complete and/or evaluable
* Demonstration of efficacy that would warrant stopping
* Determination that the primary endpoint has been met
* Determination of futility

In the case of concerns about safety, protocol compliance or data quality, the trial may resume once the concerns have been addressed to the satisfaction of the sponsor, HREC, RGO, funding and/or regulatory bodies.

### Continuation of therapy

Not applicable.

# Safety events and risks

Safety evaluations will be performed by recording adverse events (AEs), serious adverse events (SAEs), and by monitoring laboratory parameters, physical examinations, ECGs and vital signs. The following cardiac events will be considered:

1) Sudden death

2) Cardiac death

3) Overt HF requiring hospitalization, including acute pulmonary oedema

4) Serious arrhythmias requiring treatment

5) Conduction disturbances requiring a permanent pacemaker implantation.

The instructions contained in the NBMLHD Safety Reporting for Clinical Trials Therapeutic Goods will be followed (Appendix 6). These instructions outline the reporting mechanism for safety reporting in clinical trials involving therapeutic goods in NBMLHD. A Serious Adverse Event (SAE) form will be used to report events, as required (Appendix 8).

# Data management

## Overview

The investigators will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. The investigators will maintain adequate case histories of trial participants, including accurate case report forms (CRFs), and source documentation.

## Data collection, processing and storage

### Source data

Data will be collected directly from participants through face-to-face consultations, their medical records and dedicated electronic storage systems of the relevant cardiology and imaging departments.

### Data capture methods and storage

Study information will be captured using the data management software REDCap. REDCap is hosted on The University of Sydney infrastructure and is subject to the same security and backup regimen as other systems (e.g. the network file servers). Data is backed up nightly to a local backup server. All data transmissions between users and the REDCap server are encrypted. Regular data quality checks, such as automatic range checks, will be performed to identify data that appear inconsistent, incomplete, or inaccurate.

Access to REDCap is via a University of Sydney user account or (for external collaborators) via a REDCap user account created by the system administrator. The permissions granted to each user within each REDCap project is controlled by and is the responsibility of the project team. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. REDCap maintains an audit trail of data create/update/delete events that is accessible to project users that are granted permission to view it.

Data stored in REDCap will contain participants’ identifying information and clinical information.

Paper files – These will be stored in a lockable filing cabinet located in a restricted office. [E.g. For Nepean Hospital, this will be in the Cardiac Research Office on Level 5, South Block, Nepean Hospital. Access to the Cardiac Research Office is restricted and is always locked when unattended.]

Electronic study files will be stored in a restricted network folder, accessible only to approved researchers within the Cardiology department.

### Record retention

At the completion of the study, data will be kept for 15 years in a restricted department folder within the NBMLHD network. The data will be password-protected and identifying information will be separated from clinical information. Paper files will be archived and disposed of in accordance with NBMLHD policy. Professor Negishi or his subsequent delegate will be the data custodian.

# Study oversight

## Governance structure

### Trial management group (TMG)

The Site Principal Investigator is responsible for supervising any individual or party to whom they have delegated tasks at the trial site. A small group will be responsible for the day-to-day management of the trial and will include at a minimum the Site PI and project manager/research nurse/trial coordinator. The group will closely review all aspects of the conduct and progress of the trial, ensuring that there is a forum for identifying and addressing issues. Minutes will be taken at meetings, attendees listed, pertinent emails retained and phone calls documented.

### Trial steering committee (TSC)

A TSC will be established to provide expert advice and overall supervision, and ensure that the trial is conducted to the required standards. The TSC will meet at least annually, with more frequent meetings as needed. The TSC will be comprised of the Associate Investigators from each of the three sites.

### Safety monitoring

Safety oversight will be under the direction of an independent Data Safety Monitoring Board (DSMB). The DSMB will operate within agreed terms of reference / approved charter and will provide input to the Investigator.

The independent DSMB will meet twice a year via teleconference or face-to-face. It will be comprised of one interventional cardiologist, one imaging cardiologist and one biostatistician. The DSMB will report back to the Principal Investigator, i.e. Prof Negishi. Prof Negishi will ensure that the DSMB closely monitors the time it takes from admission to PCI for the duration of the trial.

## Site monitoring

Monitoring for this trial will be performed centrally by the Trial Steering Committee, after every block of 50 participants have been recruited. We will review 30% of original signed consent forms, trial eligibility data and data related to primary outcome, safety and other key data variables; all withdrawals from trial treatment and/or trial follow up; and targeted review of other data.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

## Quality control and quality assurance

Both the Investigator and Site Investigator have responsibilities in relation to quality management.

The Investigator will develop procedures that identify, evaluate and control risk for all aspects of the trial, e.g. trial design, source data management, training, eligibility, informed consent and adverse event reporting. The Investigator will also implement quality control (QC) procedures, which will include the data entry system and data QC checks. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

As outlined in the previous section (Site Monitoring), the trial monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, good clinical practice and applicable regulatory requirements.

In the event of non-compliance that significantly affects human participant protection or reliability of results, the Sponsor-Investigator will perform a root cause analysis and corrective and preventative action plan.

In addition, each clinical site will perform internal quality management of trial conduct, data and biological specimen collection, documentation and completion. An individualised quality management plan will be developed to describe a site’s quality management.

A delegation log will be used to list the research staff and clinicians involved in the study and their roles (Appendix 7).

# Statistical methods

## Sample size estimation

The primary outcome for the study is the infarct size at Day 4±2 days post PCI. Based on our pilot data, we expect an infarct size of 10.0% in the control arm, and 17.4% in the sonothrombolysis intervention arm, with a within group standard deviation of 9.9%. Using these results, we would require a total of 90 patients (45 per arm) in order to achieve 90% power at 5% significance. Anticipating 20% non-compliance/exclusion and 5% loss to followup, we plan to recruit 150 patients in total (75 per arm).

## Population to be analysed

The intention to treat population will be used in the analysis. Participants will be compared according to the group to which they were randomly allocated, regardless of compliance, crossover, or withdrawal from the trial.

## Methods of analysis

Descriptive statistics will be used to compare participants’ baseline characteristics between the three groups. Parametric and non-parametric tests will be used to compare the three study arms, depending on the distributions of the quantitative variables. Sizes of myocardial infarction and microvascular obstruction will be compared using ANOVA and multivariable regression. The chi-square test or logistic regression analysis will be used for variables with categorical endpoints. A P-value of less than 0.05 will be considered statistically significant for all analysis. Ongoing consultation with the NBMLHD biostatistician will be conducted.

## Interim analyses

### Interim monitoring of the primary study endpoint

Formal interim analysis of outcomes will be performed when approximately 50% (n=75) of patients have completed 6-month follow-up, respectively. We will use an O’Brien-Fleming monitoring boundary (truncated at 3 standard deviations) to assess whether the interim results are sufficient to indicate the futility of sonothrombolysis with PCI. We will apply a futility monitoring rule at the time of the single interim analysis. The monitoring boundary p-values associated with the 1 interim look using the O’Brien-Fleming spending function truncated at 3.00 will be 0.0054, with the final analysis being done with p=0.0492.

### Inter-observer variability in echo volume and ejection fraction assessments

Inter-observer variability in measurements of left ventricular volumes will be determined in a subset of 20 randomly chosen subjects enrolled in the study, with measurements performed by two investigators. Comparisons between these measurements will be determined with an intra-class correlation coefficient. These same variability measurement techniques will be done to examine the inter-observer variability in infarct size and microvascular obstruction measurements.

# Ethics and dissemination

## Research ethics approval and local governance authorisation

This protocol and the informed consent document and any subsequent amendments will be reviewed and approved by the Nepean Blue Mountains Local Health District Human Research Ethics Committee (NBMLHD HREC) prior to commencing the research. A letter of protocol approval by HREC will be obtained prior to the commencement of the trial, as well as approval for other trial documents requiring HREC review.

Each participating institution will also obtain institutional governance authorisation for the research and associated HREC-approved documents. A letter of authorisation will be obtained from the RGO prior to the commencement of the research at that institution. Institutional governance authorisation for any subsequent HREC-approved amendments will be obtained prior to implementation at each site.

## Amendments to the protocol

This trial will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, trial design, participant safety, or may affect a participants willingness to continue participation in the trial is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to being implemented.

## Protocol deviations and serious breaches

All protocol deviations will be recorded in the participant record (source document) and on the CRF and will be reported to the Site Principal Investigator.

Serious breaches will be reported in a timely manner (Site Principal Investigator to report to the Investigator within 72 hours and to the Site RGO within 7 days; Investigator to review and submit to the approving HREC within 7 days).

Where non-compliance significantly affects human participant protection or reliability of results, a root cause analysis will be undertaken and a corrective and preventative action plan prepared.

Where protocol deviations or serious breaches identify protocol-related issues, the protocol will be reviewed and, where indicated, amended.

# Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples and clinical information relating to participants.

The trial protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorised third party, without prior written approval of the sponsoring institution. Authorised representatives of the sponsoring institution may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this trial. The clinical trial site will permit access to such records.

All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified only by the Participant Identification Number (SID) to maintain participant confidentiality.

Clinical information will not be released without written permission of the participant, except as necessary for monitoring by HREC or regulatory agencies.

# Participant reimbursement

Patients will not be reimbursed for taking part in the trial.

# Financial disclosure and conflicts of interest

The trial will be supported by grants received by Professor Negishi from the National Heart Foundation of Australia through the 2019 Vanguard Grant, Award ID number 102251, 2021 Vanguard Grant (106084).

# Dissemination and translation plan

We intend to present and publish the results of the study in a relevant cardiac imaging journal and conferences. In consultation with the Cardiology departments of the hospitals involved, we will discuss how the knowledge gained from this study could benefit current clinical procedures.

# Outcomes and significance

We hope that by conducting this study, we will be able to determine whether most of the benefit from sonothrombolysis for acute STEMI is from the post-PCI component. The potential impact of sonothrombolysis on improving the recovery of cardiac microvasculature function may lead to change clinical guidelines based on better outcomes for this clinical population. In addition, the future implication of being able to provide sonothrombolysis in clinical centres with limited access to a catheterisation laboratory may lead to further benefits for patients as sonothrombolysis can be delivered without catheterization laboratory.

# Appendix

Appendix 1 Definity® Product information pamphlet

Appendix 2 Patient information and consent form (PICF)

Appendix 3 Master coding sheet

Appendix 4 Data collection sheet

Appendix 5 Form for Withdrawal of Participation

Appendix 6 NBMLHD Safety Reporting for Clinical Trials Therapeutic Goods

Appendix 7 Signature and Delegation log

Appendix 8 SAE form

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