



**A randomised, double blind, placebo-controlled,
parallel group Trial of low-dose adjunctive
aTeplase during prIMary PCI**

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PROTOCOL APPROVAL

A randomised, double blind, placebo-controlled, parallel group, dose-ranging Trial of low-dose adjunctive alteplase during prIMary PCI (T-TIME)

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ABBREVIATIONS

ACUITY	A Randomised Comparison of Angiomax (Bivalirudin) Versus Heparin (Unfractionated Heparin or Enoxaparin) in Patients Undergoing Early Invasive Management for Acute Coronary Syndromes Without ST-Segment Elevation
AE	Adverse event
AAR	Area-at-risk
ASSENT	Assessment of the Safety and Efficacy of New Thrombolytic Regimens
BARC	Bleeding Academic Research Consortium
BMI	Body mass index
CAD	Coronary artery disease
CMR	Cardiac magnetic resonance
CE-SSFP	Contrast-enhanced steady-state free precession
CRF	Case report form
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
Fg	Fibrinogen
FgDPs	Fibrinogen breakdown products
GG&C	NHS Greater Glasgow and Clyde
GJNH	Golden Jubilee National Hospital
gpIIbIIIa	Glycoprotein IIbIIIa inhibitor therapy
GLM	General linear model
GP	General Practitioner
GRI	Glasgow Royal Infirmary
GUSTO	Global Utilisation of Streptokinase
HF	Heart failure
HLA	Horizontal long axis
IDMC	Independent Data Monitoring Committee
ICH GCP	International Conference on Harmonization of Good Clinical Practice
IMP	Investigational Medicinal Product
IPTE	Intra-Procedure Thrombotic Events
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LV	Left ventricle
LVH	Left ventricular hypertrophy
LVEF	Left ventricular ejection fraction
LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume

MedRA	Medical dictionary for regulatory activities
MI	Myocardial infarction
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MVO	Microvascular obstruction
NT-proBNP	N-terminal pro-brain natriuretic peptide
QEUH	Queen Elizabeth University Hospital
QoL	Quality of life
PCI	Percutaneous coronary intervention
PT	Preferred term
PV	Pharmacovigilance
REC	Research Ethics Committee
SAE	Serious adverse event
SA	Short Axis
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SOT	Systemic organ term
STEMI	ST elevation myocardial infarction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIA	Transient ischaemic attack
TMG	Trial Management Group
T-TIME	A randomised, double blind, placebo-controlled, parallel group, dose ranging Trial of low-dose adjunctive alteplase during primary PCI
TIMI	Thrombolysis In Myocardial Infarction
TSC	Trial Steering Committee
UK	United Kingdom
ULN	Upper Limit of Normal
VLA	Vertical long axis

STUDY SYNOPSIS

Title of Study:	A randomised, double blind, placebo-controlled, parallel group, <u>T</u> rial of low-dose adjunctive alteplase during pr <u>I</u> Mary PCI - T-TIME
Duration of Study:	3 years
Primary Objective:	To determine the safety and efficacy of reduced doses (10 mg and 20 mg) of intra-coronary alteplase compared with placebo as an adjunct to PCI in reducing MVO and its consequences in high risk patients with STEMI.
Secondary Objective:	<p>Mechanistic:</p> <p>To explore mechanisms associated with any beneficial effects of reduced doses of alteplase.</p> <p>Safety:</p> <p>To determine the rates of adverse events associated with reduced doses of alteplase administered directly into the coronary artery as an adjunct to PCI.</p>
Primary Endpoint:	Amount of MVO (% of LV mass) disclosed by late (10 - 15 min) gadolinium contrast enhancement MRI 2 -7 days post-MI.
Rationale:	<p>Patients with acute STEMI who present with a blocked coronary artery and/or an artery with a heavy thrombus burden are at increased risk of developing heart failure. This trial aims to enrol patients with a heavy coronary thrombus burden at initial angiography to test the hypothesis that a therapeutic strategy involving reduced dose alteplase given early after coronary reperfusion as a single dose will both prevent and treat distal microvascular thrombosis and MVO. The trial aims to determine the lowest effective dose of alteplase in reducing MVO.</p> <p>Standard care with primary PCI does not involve alteplase, therefore, the following three arm design is adopted where the alteplase or placebo will be administered at the start of the PCI procedure:</p> <p>Control Arm: placebo Arm A: alteplase 10mg Arm B: alteplase 20mg</p>

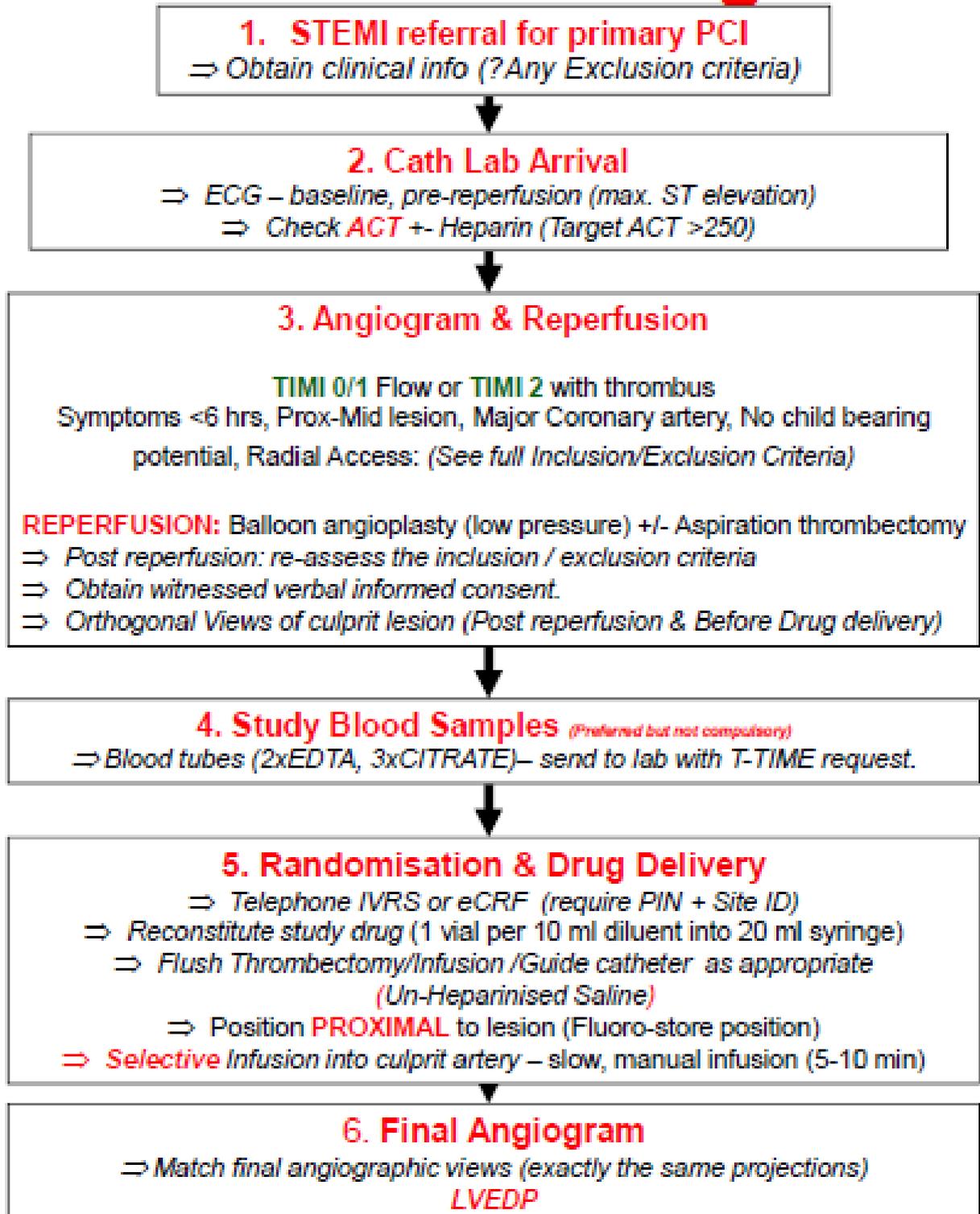
	<p>The rationale for administering low dose fibrinolytic therapy into the culprit coronary artery at the start of primary PCI (i.e. immediately after coronary reperfusion) is to <i>reduce</i> MVO, infarct size and the future risk of HF.</p> <p>Since alteplase has a 'deep tissue' half-life of up to 40 minutes, effective local thrombolysis during the procedure, alteplase is intended to <i>treat</i> and <i>reduce</i> persistent MVO at that time.</p>
Methodology:	A double-blind, randomised, parallel group, placebo-controlled dose ranging clinical trial. A Phase II therapeutic exploratory trial including comparison with the standard treatment regimen.
Sample Size:	<p>618 STEMI patients (n=206/group; placebo, alteplase 10 mg, alteplase 20 mg)</p> <p>A sample size of 618 (minimum 186/group) would result in 80% and 90% power to detect between-group mean differences of 1.49% or 1.72% respectively assuming mean (SD) of 3.2 (5.1)% for the extent of late MVO in the comparator group.</p> <p>With 186 randomised patients in each group, or 558 subjects randomised in total, if the proportion of patients in the control (placebo) group with LATE MVO is 47% then a 15% absolute difference in the rate of LATE MVO (i.e. 32% of patients with MVO) in the alteplase group could be detected with 80% power at a 5% level of significance. 618 (n=206 / group) patients will be recruited to allow for incomplete data.</p>
Screening	All primary PCI referrals to the participating hospitals
Registration/Randomisation:	<p>Administered via the Robertson Centre for Biostatistics in 1:1:1 proportion of:</p> <p>Control Arm: placebo</p> <p>Arm A: alteplase 10mg</p> <p>Arm B: alteplase 20mg</p> <p>A kit will be allocated at the time of randomisation via IVRS/IWRS.</p>
Main Inclusion Criteria:	Males aged ≥18 years; females ≥18 years not of child bearing

	<p>potential</p> <p>Acute myocardial infarction (symptom onset \leq 6 hours) with persistent ST segment elevation or recent left bundle branch block</p> <p>Coronary artery occlusion (TIMI coronary flow grade 0 or 1)</p> <p>or</p> <p>Impaired coronary flow (TIMI flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2+).</p> <p>Proximal-mid culprit lesion location in a major coronary artery (i.e. the right, left anterior descending, intermediate or circumflex coronary artery)</p> <p>Radial artery access</p>
<p>Main Exclusion Criteria:</p>	<p>Shock (systolic blood pressure $<$ 90 mmHg with clinical signs of peripheral hypoperfusion despite adequate filling)</p> <p>Normal coronary flow grade (TIMI flow grade 3) at initial angiography</p> <p>Functional coronary collateral supply (Rentrop grade 2/3) to the culprit artery</p> <p>Multivessel PCI intended before the day 2 -7 MRI scan</p> <p>Non-cardiac co-morbidity with expected survival $<$1 year</p> <p>Estimated body weight $<$ 60 kg</p> <p>Contra-indication to contrast-enhanced MRI</p> <p>Pacemaker</p> <p>Implantable defibrillator</p> <p>eGFR $<$30ml/min/1.73m²</p> <p>Previous infarction in the culprit artery (known or suspected clinically, e.g. wall motion abnormality revealed by echocardiography)</p> <p>Significant bleeding problem either at present or within the past 6 months</p> <p>Patients with current concomitant oral anticoagulant therapy (INR $>$1.3), including apixaban, dabigatran, and rivaroxaban</p> <p>Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)</p> <p>Known haemorrhagic diathesis</p> <p>Severe uncontrolled hypertension $>$180/110 mmHg not</p>

	<p>controlled by medical therapy</p> <p>Major surgery, biopsy of a parenchymal organ, or significant trauma within the past 2 months (this includes any trauma associated with the current STEMI)</p> <p>Recent trauma to the head or cranium (< 2 months)</p> <p>Prolonged cardiopulmonary resuscitation (> 2 minutes) within the past 2 weeks</p> <p>Acute pericarditis and/or subacute bacterial endocarditis e.g. valve mass or vegetation revealed by echocardiography</p> <p>Acute pancreatitis</p> <p>Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis</p> <p>Arterial aneurysm and known arterial/venous malformation</p> <p>Neoplasm with increased bleeding risk</p> <p>Any known history of haemorrhagic stroke or stroke of unknown origin</p> <p>Known history of ischaemic stroke or transient ischaemic attack < 6 months</p> <p>Dementia</p> <p>Hypersensitivity to gentamicin</p> <p>Women of child-bearing potential (i.e. pre-menopause) or breast feeding</p> <p>Previous randomization to this study or participation in a study with an investigational drug or medical device within 90 days prior to randomisation</p> <p>Incapacity or inability to provide informed consent</p> <p>Requirement for immunosuppressive drug therapy at any time during the past 3 months; whether administered orally, subcutaneously or intravenously. This would include corticosteroids (but not inhaled or topical), drugs used following transplantation (e.g. tacrolimus, cyclosporine), anti-metabolite therapies (e.g. mycophenolic acid (Myfortic), azathioprine, leflunomide (Arava)), and immunomodulators including biologics (e.g. adalimumab (HUMIRA), etanercept (Enbrel), aldesleukin), and DMARDS (cyclophosphamide, methotrexate, etc). Please note this list is not exhaustive and a requirement for other immunosuppressive drugs not listed would also exclude the patient.</p>
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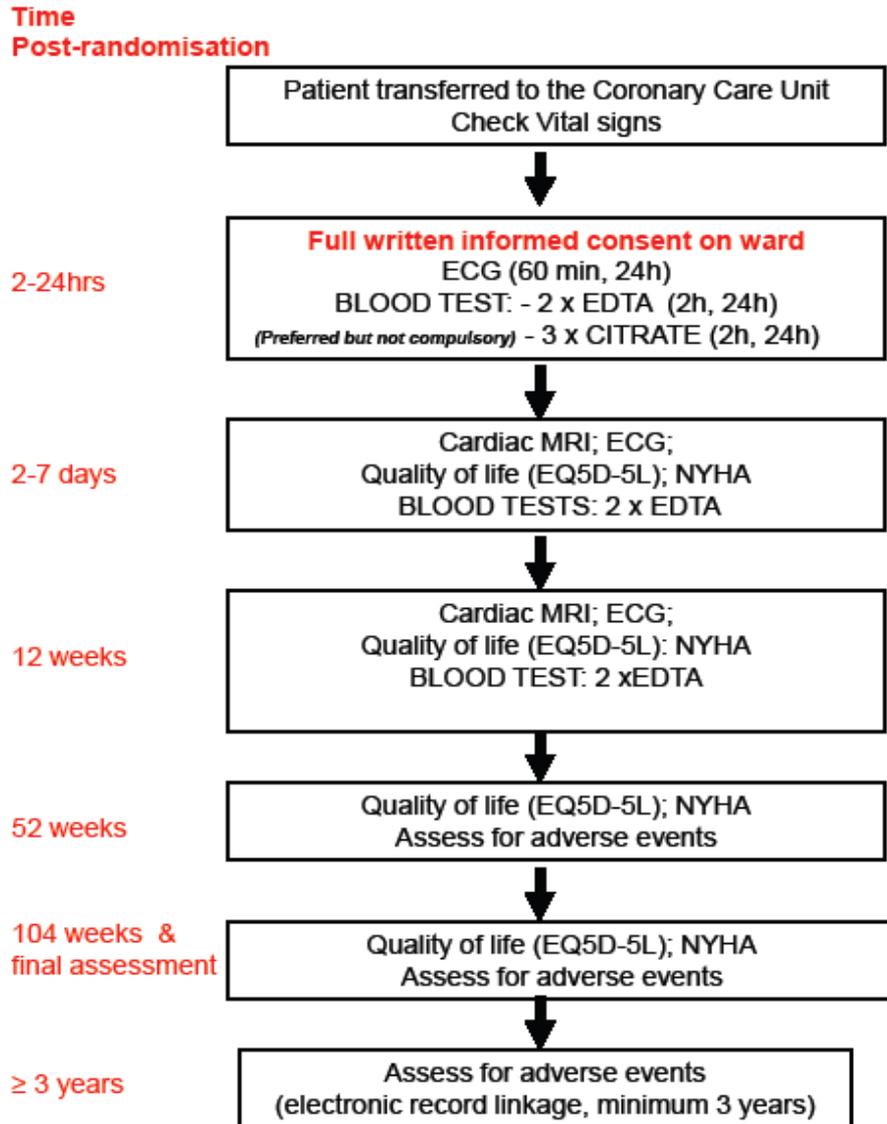
	<p>Active or prophylactic treatment with oral or parenteral antibiotic, antifungal or antiviral therapy to prevent or treat infection.</p> <p>Any anti-cancer treatment (excluding surgery as this is covered above) at any time during the past 3 months including chemotherapy, radiotherapy and treatment with biologics such as Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitors (e.g. bevacizumab, pazopanib). This list is not exhaustive and sponsor or CI should be contacted for advice if required.</p>
Product, Dose, Modes of Administration:	Eligible patients will be allocated to placebo, 10 mg of alteplase or 20 mg of alteplase after reperfusion. Kits will contain two identical vials of alteplase 10mg and/or placebo as a powdered lyophilisate. Diluent will also be supplied. The reconstituted drug will be administered at the start of the procedure. Each patient will receive the reconstituted alteplase/placebo solution as a slow manual infusion via a flushed catheter for local intracoronary infusion (e.g. aspiration catheter, perfusion catheter) over a period of 5 -10 minutes.
Duration of Treatment:	Single treatment consisting of a single slow infusion administration after reperfusion with either aspiration thrombectomy or angioplasty but before stenting during primary PCI
Statistical Analysis	A Chi squared test with logistic regression will be used to calculate confidence intervals. Since there are 3 groups (placebo, alteplase 10mg, alteplase 20mg), we will first compare alteplase 20 mg vs. placebo with $p < 0.05$ required for significance. If this test is significant we will then test alteplase 10 mg vs. placebo. The Robertson Centre for Biostatistics will manage and analyse trial data. All of the statistical analyses will be conducted according to the Statistical Analysis Plan, which will be authored by the Trial Statistician and agreed by the Trial Steering Committee. Long-term follow-up analysis (i.e. a minimum of 3 years after randomisation) will be conducted if funding is obtained for this.

T-TIME Flow Diagram



Note: Points 1-3 reflect optimal standard of care

T-TIME Post-PCI



CITRATE: Coagulation
EDTA: Troponin T; NT-proBNP

SCHEDULE OF ASSESSMENTS

Study timeline ¹	Pre-randomisation	T ₀	0-2 hrs Procedure	2 hrs (±1hr)	24 hrs (±12hr)	Day 2-7 ²	12 (±2) wks	52 (±4) wks 104 (±4) wks Final assessment ⁴	3 years
Assessment	Catheter laboratory		Cath lab or CCU	CCU	CCU	CCU/Ward/MRI	Outpatient/MRI	Outpatient	Record linkage
Obtain verbal informed consent	√								
Obtain written informed consent **					√ ³				
Primary PCI	√								
Review Inclusion/Exclusion Criteria	√								
Vital sign observation	√	√	√	√	√	√			
Angiographic measurements			√						
Electrocardiogram	√		√	√	√	√	√		
Randomisation		√							
Trial drug administration		√							
Physical Examination	√		√						
Weight and Height	√								
Complete Blood Count & ACT check	√				√				
Blood chemistry (i.e. U&E)	√				√				
Medical/Disease History	√			√					
Drug therapy	√			√		√			

Study timeline ¹	Pre-randomisation	T ₀	0-2 hrs Procedure	2 hrs (±1hr)	24 hrs (±12hr)	Day 2-7 ²	12 (±2) wks	52 (±4) wks 104 (±4) wks Final assessment ⁴	3 years
MRI						√	√		
Troponin-T		√ ⁵		√ ⁵	√ ⁵	√	√		
NT-proBNP		√ ⁵		√ ⁵	√ ⁵	√	√		
COAGULATION		√ ⁵		√ ⁵	√ ⁵				
EQ5D-5L						√	√	√	
Feasibility questionnaire			√						
Sub-study: Thrombus histopathology		√							
Sub-study: Coronary physiology			√						
Sub-study: Optical coherence tomography			√						
Follow-up contact (telephone/letter/clinic review as required)								√	
Adverse events evaluation and reporting		√	√	√	√	√	√	√	

Study timeline ¹	Pre-randomisation	T ₀	0-2 hrs Procedure	2 hrs (±1hr)	24 hrs (±12hr)	Day 2-7 ²	12 (±2) wks	52 (±4) wks 104 (±4) wks Final assessment ⁴	3 years
Long term follow up with electronic record linkage								√ (close-out at 30 months)	√ (min 3 years)

1 From time of study treatment administration

2 All assessments to be completed on day 2 where possible. If necessary assessments may be completed on different days to accommodate scheduling

3 Full informed consent should be obtained within 24 hours of admission or prior to hospital transfer if this is earlier. When written consent is not possible e.g. the patient is unwell, then consent may be delayed and obtained when this becomes feasible. No further scheduled study assessments can be performed until full informed consent is obtained.

4 The time-point for the final assessment is defined as when the last randomised participant has completed 12 months follow-up.

5 Research blood tests at times 0, 2 hrs and 24 hrs are preferred but not compulsory.

1. INTRODUCTION

1.1 Background

Acute ST elevation myocardial infarction (STEMI) is a leading global cause of premature morbidity and mortality (1). Even though primary percutaneous coronary intervention (PCI) saves lives, nearly one half of STEMI patients have sustained impairment of microcirculatory blood flow, even after re-opening the culprit coronary artery (2-4). When detected clinically, this phenomenon is termed microvascular obstruction (MVO) (2-4).

Atherothrombosis is one of four pathological contributors to microcirculatory damage in the infarcted human heart (5-10) and post-mortem studies have confirmed that fibrin deposition contributes to MVO (7). In addition, microthrombi have a mixed composition involving both platelets (pale white) and fibrin (dark red) (7,10,11). Therefore, optimal pharmacological clot degradation may be best achieved with a combined approach with fibrin-specific lytics and anti-platelet drugs. Microthrombi may arise by distal microembolisation or local microvascular thrombosis (7-17) including through vessel plugging with reperfusion injury (17).

While some studies have found that thrombus aspiration from within the lumen of the artery reduces infarct size, increases myocardial salvage (16,17) and improves clinical outcomes (18), other studies have not confirmed these results (19,20). Previously, thrombus aspiration had a Class IIa recommendation in clinical guidelines (i.e. should be considered) (1) based on Level B evidence from one single-centre randomised trial (18). Following publication of the large TASTE (20) and TOTAL (21) trials, which found that routine use of aspiration thrombectomy was not associated with benefit, international practice guideline recommendations changed ((22) 31 March 2016). Routine aspiration thrombectomy in all-comers is not recommended (Class III) and a strategy of selective or bailout thrombectomy may/might be recommended however due to insufficient data the effectiveness is not well established (Class IIb) (22). When compared with placebo (INFUSE-AMI, 20) or intravenous administration (AIDA-STEMI, 23), intra-coronary anti-platelet therapy with glycoprotein IIb/IIIa inhibition is not associated with convincing effects on surrogate (MVO, infarct size, LV function) or clinical outcomes. Prevention of thrombus embolisation with distal protection devices has also not translated into clinical benefits (1,24), and other device-based approaches for prevention of no-reflow, such as with mesh-covered stents (25), continue to be actively investigated. Overall, there is no evidence-based device- or pharmacological therapy for MVO (24) and in a recent Editorial (11), an internationally renowned North

American pathologist drew attention to "lytic agents" as a potential new therapeutic option for MVO.

1.2 Rationale for reduced dose alteplase as an adjunct to primary PCI

Primary PCI is a multi-step procedure. The first step initially involves coronary angiography to reveal coronary anatomy and the culprit lesion implicated in the acute MI. Next, coronary reperfusion may be achieved using a thin (0.014") coronary guidewire followed by balloon angioplasty and/or selective thrombus removal by transcatheter aspiration thrombectomy. Thirdly, the standard of care procedure involves stenting and then optimisation of the stent result by high-pressure balloon angioplasty within the stent.

Thrombolytic drugs activate endogenous fibrinolysis (26). While systemic thrombolysis with full dose weight adjusted fibrin-specific (2nd& 3rd generation) lytic drugs is an effective treatment for STEMI (1), this approach is not recommended before or during primary PCI because of the increased risk of bleeding (1). However, there is some evidence to suggest that delivery of anti-fibrinolytic therapy *during PCI* directly into the coronary artery following mechanical reperfusion may reduce the incidence of MVO. Sezer *et al* (27) demonstrated that the delivery of 250 U streptokinase at the end of primary PCI in 41 STEMI patients improved myocardial reperfusion when compared with placebo. However, this study had some limitations:

- The improvement in perfusion did not translate into improvements in long-term ventricular size or function
- Streptokinase is not selective for fibrin and it has a higher risk of bleeding than fibrin-specific thrombolytics such as alteplase and tenecteplase (26)
- Transcatheter aspiration of thrombus was not performed
- Streptokinase was given through the guide catheter rather than local delivery of the lytic drug directly into the culprit artery. Drug administration into the guide catheter means that the thrombolytic would be dispersed into coronary branches not connected to the culprit artery, in other words, drug delivery was to non-relevant coronary arteries potentially reducing drug exposure to fibrin-rich thrombus in the culprit artery microcirculation
- Since streptokinase was given after the end of the PCI procedure, thrombolysis could only treat established microvascular thrombosis rather than prevent its occurrence by being given earlier during PCI (as is proposed in our study).

These problems may explain why Sezer's findings have not been adopted into clinical practice. There are only two other studies (n<40 patients (27,28)) neither of which could inform clinical practice, underlining the need for more research.

Sezer's findings indicate that intra-coronary thrombolysis has the potential to reduce microvascular damage during primary PCI. Our proposal will use alteplase, a more potent fibrin-specific second generation lytic drug than streptokinase and comparable potency with 3rd generation drugs such as reteplase (26); accordingly, our trial will determine whether or not alteplase will reduce microembolic thrombus and improve microvascular function when delivered locally (11).

1.3 Alteplase: prior experience and dose selection

Alteplase is a fibrin-specific 2nd generation plasminogen activator and thrombolytic drug (26,30). Full standard dose alteplase (100 mg) is widely used world-wide as a primary reperfusion therapy for STEMI in hospitals where primary PCI is not available.

The main difference between alteplase and 3rd generation lytic drugs is that alteplase has a circulating half life of about 5 min (hepatic metabolism) so is administered as an intravenous infusion over 90 min whereas 3rd generation thrombolytics such as reteplase and tenecteplase have longer half lives (i.e. 15 min) and so can be administered as a single bolus infusion (26, 30). However, although alteplase has a shorter circulating half life the deep tissue beta half life of alteplase is 40 min (30) and this pharmacokinetic property is very relevant for local drug delivery into the coronary microcirculation. The differences between clearance of alteplase from blood and deep tissues are because alteplase is metabolised in the liver, meaning that when alteplase is directly administered into tissues it will persist for a much longer period of time since it does not initially pass through the liver in the blood. Compared with reteplase (31,32) and tenecteplase (33), alteplase has similar efficacy for restoring normal (TIMI grade III) coronary artery blood flow. Fibrinolytic drugs also have procoagulant effects (24,34,35) however haemostatic effects such as thrombin activation are similar with alteplase and reteplase (24,34,35), although pro-coagulant effects may be lower with tenecteplase due to lower activation of the kallikrein-factor XII system (24,35). Concomitant treatment with anti-thrombotic drugs (e.g. heparin, glycoprotein IIb/IIIa inhibitors, bivalirudin) attenuates the pro-coagulant effects of thrombolytic drugs (1).

The ASSENT-II (36) and GUSTO-III (37) trials were designed to compare alteplase with 3rd generation thrombolytics (tenecteplase and reteplase, respectively) based on the rationale that the latter might be easier to use in clinical practice provided their efficacy was at least equivalent to alteplase. In the ASSENT-II trial (36), in which 16,950 STEMI patients were randomised within 6 hrs of symptom onset to either front-loaded full dose alteplase (100 mg infused over 90 min) or weight-adjusted tenecteplase (~0.5 mg/kg), along with aspirin and intravenous heparin, the 30-day mortality rates in each group were similar (6.1% with alteplase and 6.2% with tenecteplase) and the pre-specified criteria for equivalence were met (36). The rates of intra-cranial and severe bleeding were similar ($p=0.15$) although blood

transfusions were slightly more common in alteplase treated patients (5.5%) than in tenecteplase-treated patients (4.3%). In the GUSTO-III trial (37), 15,059 STEMI patients were randomised to either 100 mg of alteplase or reteplase. The 30-day mortality rates (7.2% for alteplase and 7.5% for reteplase) and haemorrhagic stroke rates (0.9%) were similar. Taken together, these trials confirm that alteplase has similar efficacy and safety compared to 3rd generation drugs for thrombolysis in STEMI.

Anti-thrombotic therapy and thrombolysis: Thrombolytic drugs lyse fibrin in red thrombus but these drugs do not have anti-platelet effects and so have no effect on platelet-rich 'white' clot. Thrombolytic drugs are also associated with pro-coagulant effects including platelet activation (34,35). For these reasons, optimal anti-platelet therapy and anti-coagulation during and after thrombolysis are essential (1). In the ASSENT-IV trial, full dose thrombolysis prior to primary PCI with stents in the absence of glycoprotein IIb/IIIa inhibitors was associated with an increased risks of re-infarction (possibly because of platelet activation) and bleeding (38). The ASSENT-IV investigators concluded that sub-optimal anti-thrombotic therapy, including the absence of heparin after the initial 5000 IU bolus, the lack of an up-front loading dose with clopidogrel, and the prohibition of glycoprotein IIb/IIIa inhibitor therapy (except in bailout circumstances) contributed to the early thrombotic complications (i.e. recurrent myocardial infarction) observed in the tenecteplase-treated patients.

Clinical trials of primary PCI with reperfusion facilitated by giving full dose thrombolysis during transfer prior to PCI ('facilitated primary PCI') have been negative (including MVO outcomes in some of the trials) such as ASSENT-IV (38) and LIPSIA-STEMI (39). In FINESSE (40), there was an improvement in microcirculation indices in the half dose reteplase / abciximab group. In this case, any effect of reteplase to enhance platelet activation may have been attenuated by concomitant anti-platelet therapy with abciximab. Overall, the reasons for why facilitated PCI trials were unsuccessful may include (1) treatment delays with PCI associated with lytic therapy vs. usual care (nearly 30 min difference in LIPSIA, which is a very substantial prolongation in ischaemic time), (2) inadequate anti-coagulation and anti-platelet therapy, (3) harmful bleeding in STEMI patients with femoral artery access (at times, a non-compressible puncture site), and (4) earlier trials used streptokinase (a non-fibrin specific lytic with 'off target' effects). In LIPSIA (39), only 29% of thrombolysed patients received optimal anti-platelet therapy with glycoprotein IIb/IIIa inhibitors. The LIPSIA investigators recognized that anti-platelet therapy (to minimize bleeding risk in full dose thrombolysis) had been inadequate in their trial and that thrombolysis prior to PCI had prolonged the ischaemic period in the facilitated group of patients. The LIPSIA investigators specifically referred to the potential value of reduced dose thrombolysis with optimal anti-platelet therapy, which is what we propose in T-TIME, uniquely in a dose ranging trial with intra-coronary lytic administration after reperfusion.

In the HORIZONS trial which involved STEMI patients treated with primary PCI, compared with standard treatment with glycoprotein IIb/IIIa inhibitors, bivalirudin reduced the risk of bleeding (including access site and non-access site bleeds) but was also associated with an increased risk of stent thrombosis (41), as has been reported in subsequent clinical trials including EUROMAX (NCT01087723; PMID24171490) and HEAT-PCI (NCT01519518; PMID25002178) (42). In HEAT-PCI, glycoprotein IIb/IIIa inhibitor therapy was used for 'bail-out' in line with clinical guideline recommendations (1) and the rate of glycoprotein IIb/IIIa was 15% in the heparin group and 13% in the bivalirudin group. In HEAT-PCI (42), the primary efficacy outcome (a composite of all-cause mortality, cerebrovascular accident, reinfarction, or unplanned target lesion revascularisation) occurred in 79 (8.7%) of 905 patients in the bivalirudin group and 52 (5.7%) of 907 patients in the heparin group (absolute risk difference 3.0%; relative risk [RR] 1.52, 95% CI 1.09–2.13, $p=0.01$). The primary safety outcome was the incidence of major bleeding (type 3–5 as per Bleeding Academic Research Consortium definitions) and it occurred in 32 (3.5%) of 905 patients in the bivalirudin group and 28 (3.1%) of 907 patients in the heparin group (0.4%; 1.15, 0.70–1.89, $p=0.59$) (42). (42). Currently, clinical opinion in NHS hospitals, including the consensus from investigators in this trial, increasingly favours unfractionated heparin with bail-out gpIIb/IIIa inhibitor therapy rather than bivalirudin. Evidence-based optimal dual anti-platelet therapy includes aspirin with clopidogrel (with a high loading dose of 600 mg), prasugrel, or ticagrelor (1). Currently, there is no consensus across the NHS for a high loading dose with clopidogrel, prasugrel or ticagrelor, although increasingly prasugrel and ticagrelor are preferred (1). Therefore in T-TIME, optimal and standardised anti-thrombotic management will begin at the first medical contact and include dual anti-platelet therapy with aspirin and an initial loading dose of clopidogrel, ticagrelor or prasugrel (according to local hospital practices) and at least 5000 IU of unfractionated heparin (70U/kg) (1). On arrival in the cardiac catheter laboratory anti-coagulation will be verified with a check of the activated clotting time (ACT) with additional heparin (70 U/kg) given as clinically appropriate (1). If bivalirudin is used the drug should be continued after the PCI at the standard dose until the patient has returned to the ward for up to 4 hours in line with contemporary practice.

1.3.1 Evidence base in relation to SAFETY (systemic effect) and EFFICACY for very low dose alteplase ~ 10 mg (1/10th standard dose, 100 mg)

Sezer *et al* (25) used 250,000 units of streptokinase which is one sixth of the usual dose given intravenously in acute MI (1.5 million units). Given streptokinase is much less effective than alteplase, we believe that using a lower equivalent dose of alteplase, such as one tenth of the standard dose is an appropriate strategy. The licensed thrombolytic dose for a person > 65 kg is 100 mg of alteplase. We have therefore adopted a 10-fold lower dose as the lowest dose of alteplase in this study.

1.3.1.1 Safety of 10 mg of alteplase

The main safety concern with thrombolytic drugs such as alteplase is bleeding which may occur at the site of vascular access or systemically. The risk of bleeding is dose dependent (43) and the usual systemic dose is weight-adjusted.

Low dose alteplase (e.g. 2 - 4 mg) is widely used in hospitals worldwide to recanalise central venous catheters occluded with thrombus and this treatment approach has been evaluated with a systematic review (44). The 10 mg loading dose is the standard initial step with systemic thrombolysis for acute MI (1).

Reduced dose alteplase has been described in 3 PCI studies although none of these studies involved a dose of 10 mg (45-47).

1.3.1.2 Efficacy of 10 mg alteplase

In TIMI 14 (45), reperfusion treatment regimens consisting of total alteplase doses of 20, 35, 50, or 65 mg produced 90-minute TIMI 3 flow rates that were at least comparable to those observed with full-dose alteplase alone. Higher rates of TIMI 3 flow at both 60 and 90 minutes were observed with increasing duration of administration of alteplase, progressing from a bolus alone to a bolus followed by either a 30- or 60-minute infusion ($P < 0.02$). In the study by Maioli *et al* (46), an 'open artery' with TIMI grade III flow at initial angiography was present in 44% of patients pre-treated with 20 mg of intravenous alteplase compared to 1% of patients who did not receive alteplase. The improvement in left ventricular ejection fraction at 1 month post-MI was greater in patients who had TIMI grade III coronary flow at initial angiography compared to patients who did not ($P = 0.005$). In the study by Gurbel *et al* (47) lesion-directed therapy with 20 mg of alteplase reduced the mean thrombus score from 2.2 to 1.6 ($p = 0.02$). The greatest benefit was observed in patients with a heavy thrombus burden (TIMI thrombus grade ≥ 2). In contrast to contemporary primary PCI with stents, only 6% of patients in this study received a stent and the effects of alteplase on systemic fibrinolysis were still evident 4 hrs afterwards.

In their clinical study, Kelly *et al* (29) used the fibrin-specific agent, tenecteplase, at 1/10th standard dose (5 mg) and found it to be successful at dissolving angiographic thrombus and/or improving flow. In our experience in the Golden Jubilee National Hospital, reduced dose intra-coronary alteplase (10 – 20 mg) has been administered on clinical grounds in selected cases with improvements in coronary flow grade and without complication. The ATHENS trial (48) was a placebo-controlled trial of reduced-dose (10 mg; 1/5th standard dose) tenecteplase given before primary PCI in 284 patients presenting with STEMI. Bleeding complications tended to be higher in the tenecteplase-treated patients although the between-group difference was not statistically significant and no-cases of haemorrhagic stroke

occurred. Taken together, these data indicate that a 1/10th standard dose of a fibrin-specific thrombolytic drug should not be associated with an increased risk of bleeding, especially since femoral artery access will not be used in our trial (and femoral access is the main cause of bleeding in primary PCI). In the TIMI10A trial (43), 5mg of tenecteplase (i.e. 1/10th standard dose) had comparable efficacy to higher doses (Figure 1).

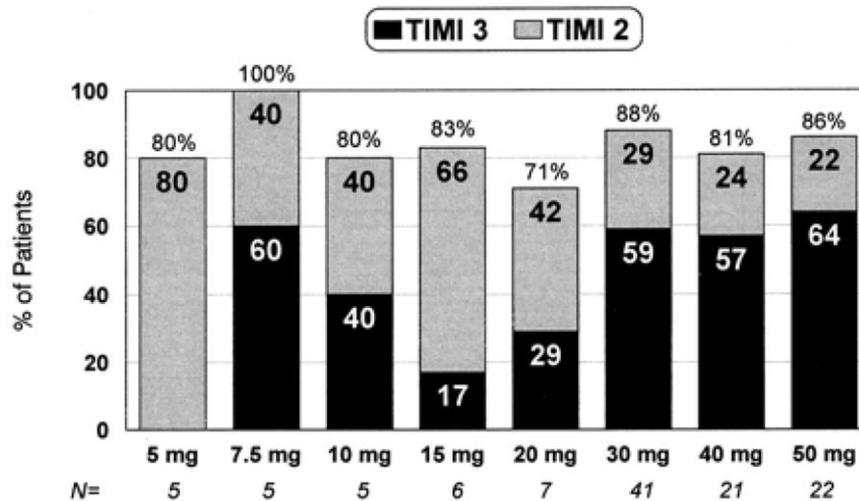


Figure 1. Coronary flow grade according to dose of tenecteplase in study participants in the TIMI 10A trial.

A similar % of patients achieved complete coronary artery reperfusion (TIMI grade ≥ 2). Based on these descriptions and that of Sezer et al (25) with a less potent thrombolytic (streptokinase, 1/6th dose), we believe that the 10mg dose (1/10th dose) of alteplase will be efficacious.

Overall, there is proof of efficacy with half dose IV alteplase (FINESSE (40)) and evidence of efficacy with intracoronary (IC) administration (47) and IC tenecteplase at 1/10th dose (29). By giving local intracoronary administration, the potential for alteplase to bind to fibrin in residual clot is maximised. A pharmacokinetic study of accelerated front loaded alteplase (15 mg loading dose IV, 100 mg IV/ 90 min) rapidly (~5 min) achieved steady state plasma concentrations that were higher than a standard infusion (49). Our design exploits the timely therapeutic effects with the front-loaded dose approach. The standard intravenous loading dose results in a high initial circulating concentration of alteplase. Locally, this is associated with improved initial fibrinolysis (culprit or microcirculatory patency). Our approach aims to recapitulate this high initial local concentration at the start of primary PCI by direct administration of the drug into the culprit artery, whilst avoiding the potentially harmful effects of high systemic concentrations of the thrombolytic drug. Impaired intracoronary perfusion during STEMI means there is a slower 'washout' of drug which we hypothesise will help *maintain* therapeutic concentrations of alteplase locally for targeting and degrading fibrin by plasminogen activation. We hypothesise that sustained local intracoronary perfusion

directly into the culprit coronary artery with therapeutic concentrations of alteplase will improve the achievement of therapeutic microvascular concentrations within the volume of tissue being treated.

The hypothesis for the mode of action of our intervention is based on initial prolonged exposure to alteplase caused by the poor perfusion created by the clot – this in turn will allow distribution of alteplase into the microvasculature of deep tissue where the half life of alteplase is ~40 min (30). Local intra-coronary administration should lead to effective plasminogen activation and fibrinolysis within the microvasculature and reduce the complications seen post PCI that may be attributable to distal microvascular obstruction.

1.3.2 Evidence base in relation to SAFETY (systemic effect) and EFFICACY for low dose alteplase ~ 20 mg (1/5th standard dose, 100 mg)

Reduced dose (20 mg) alteplase has been described in 3 PCI studies. In the TIMI 14A trial (45), 888 STEMI patients who had presented <12 hours from onset of symptoms were treated with aspirin and randomized initially to either 100 mg of accelerated-dose alteplase (control) or abciximab (bolus 0.25 mg/kg and 12-hour infusion of 0.125 µg/kg/min) alone or in combination with reduced doses of alteplase (20 to 65 mg) or streptokinase (500 000 U to 1.5 MU). Reperfusion treatment regimens consisting of total alteplase doses of 20, 35, 50, or 65 mg produced 90-minute TIMI 3 flow rates that were at least comparable to those observed with full-dose alteplase alone. Higher rates of TIMI 3 flow at both 60 and 90 minutes were observed with increasing duration of administration of alteplase, progressing from a bolus alone to a bolus followed by either a 30- or 60-minute infusion (P<0.02). Based on results from the initial dose-finding phase, TIMI 3 flow rates were highest in the 50-mg alteplase plus abciximab group versus the alteplase-only group at both 60 minutes (72% vs. 43%; P=0.0009) and 90 minutes (77% vs. 62%; P=0.02). The rates of major haemorrhage were 6% in patients receiving alteplase alone (n=235), 3% with abciximab alone (n=532), 10% with streptokinase plus abciximab (n=143), 7% with 50 mg of alteplase plus abciximab and low-dose heparin (n=103), and 1% with 50 mg of alteplase plus abciximab with very-low-dose heparin (n=70).

In 212 consecutive patients, Maioli *et al* (46) undertook a non-randomised comparison of an 'out-of-hours' strategy of facilitated primary PCI which involved a reduced dose of alteplase (20 mg) administered as an intravenous bolus combined with abciximab during transfer before PCI compared with 'in hours' standard primary PCI without prior thrombolysis. The main difference in baseline characteristics between patients in each group was a longer time to treatment in the 'out-of-hours' patients. Femoral artery access was used in all patients and major bleeding tended to be more common in the facilitated group (6.1%) compared to the

standard primary PCI group (3.0%, P=0.23). Bleeding events were mostly associated with femoral bleeding.

Gurbelet *et al* (47) undertook an open-label prospective study of intra-coronary thrombolysis with reduced dose alteplase (20 mg) in 51 patients with unstable angina and coronary thrombus. Intra-coronary drug administration involved a 'Tracker' delivery catheter. Forty five patients received lesion-directed therapy and 6 did not because this relatively inflexible catheter could not be passed into the coronary artery. The no-reflow phenomenon did not occur in any patients. One patient had a 'mini-stroke' with no residual neurological deficit. Moderate or severe bleeding occurred in 24% of patients but the majority (70%) of these events were related to femoral access bleeding complications.

In the Golden Jubilee National Hospital, intra-coronary alteplase (10 – 20 mg) for local thrombolysis of heavy clot burden during emergency PCI was administered on clinical grounds in selected cases, one of which had a history of an ischaemic stroke 10 months earlier. No complications occurred. Bleeding is a major cause of morbidity and mortality after primary PCI (1, 50) and for this reason our strategy is focused on avoiding bleeding complications. Femoral artery access is strongly associated with bleeding after primary PCI (51). Since radial artery access (a compressible site) will be used rather than femoral access, low dose intra-coronary alteplase during primary PCI should not be associated with a higher rate of bleeding overall.

Rationale for 10mg vs. 20mg of alteplase

Since *safety is our primary concern*, we are motivated to determine the lowest effective intracoronary (IC) dose of alteplase and for this reason we have adopted a *dose ranging study* of 10 mg of alteplase IC (1/10th standard dose) and 20 mg IC alteplase (1/5th standard IV dose). Alteplase (or placebo) will be administered at the start of PCI. In the previous studies which administered 20 mg dose of alteplase (45-47), either intravenous (45,46) or intracoronary (47), the bleeding rates were slightly higher than the rates currently observed in clinical practice. However, bleeding was substantially related to femoral artery access site problems which should be rare in our trial since radial artery access is now considered routine (1). Since locally-administered thrombolysis can be expected to achieve higher tissue concentrations than the equivalent dose administered systemically 10 mg of alteplase may optimise safety whereas the 20 mg dose of alteplase may have superior lytic effects which could translate into improved perfusion and reduced MVO overall. Based on the available safety evidence and known dose-response relationship with bleeding complications (26,43), we think it is unlikely that there will be any safety issues with either dose, especially since radial artery access is mandated for our trial.

Rationale for administration of alteplase in a single dose at the beginning of PCI

In the TIMI 14 trial (45), the efficacy of alteplase for achieving normal coronary blood flow was higher with a longer duration of intravenous infusion (60 min vs. 30 min). In primary PCI in ordinary clinical practice, an intra-coronary infusion of alteplase would be neither feasible nor safe because of the indwelling catheter. Since the deep tissue (beta) half life of alteplase is 40 min (30), a locally administered manual infusion of intra-coronary alteplase is likely to have a much longer duration of effect than an intravenous bolus alteplase ($T_{1/2} = 5$ min). Therefore, the initial dose of alteplase at the beginning of primary PCI should be effective initially during primary PCI when thrombus burden is greatest and so prevent and/or minimise MVO and because of the 'deep tissue' half life the duration of local thrombolysis may be effective even after the end of the PCI procedure (1, 37).

While there is a theoretical rationale for giving a second dose of alteplase at the end of PCI, this second time-point would require a very much larger sample size with more groups, and potentially a higher total dose of alteplase, which would call into question safety. In terms of translation to clinical practice, a single infused dose after initial reperfusion would be very straightforward to adopt in routine practice in all hospitals that provide primary PCI.

1.3.3 Significance of bleeding post-MI

Bleeding and transfusions after acute MI are adverse prognostic factors (1, 50-54) and are more frequent in the presence of concomitant health problems (54). Thrombolysis is also associated with heart muscle bleeding (55). In acute stroke, intra-arterial thrombolysis is not associated with a higher risk of cerebral haemorrhage compared to IV thrombolysis (56). Moreover, since other drugs with an evidence base in acute MI, such as aspirin and glycoprotein IIb/IIIa inhibitors, also promote bleeding (1), haemorrhage may be a sign of treatment EFFICACY. We address this uncertainty not only by using alteplase at greatly reduced doses in an open (recannalised) artery but also by studying the frequency and extent of myocardial haemorrhage in each treatment group as detected by T2*-weighted MRI methods which can specifically reveal haemorrhage (57,58).

Since the main source of bleeding in STEMI patients treated by primary PCI relates to femoral artery access (1, 50-52), in order to minimise the bleeding risk for study participants, radial artery access will be advised. The radial artery is a compressible site meaning access site bleeding can be minimised. The radial artery is standard practice for primary PCI and is used in close to 90% of all procedures.

Haemorrhage is a common undesirable effect associated with the use of full dose intravenous alteplase. Ecchymoses are observed commonly but usually do not require any specific action.

Death and permanent disability are reported in patients who have experienced stroke (including intracranial bleeding) and other serious bleeding episodes (38). In our study bleeding reactions are expected to be much less frequent than expected with full dose systemic alteplase because the study drug will be administered locally at 1/5th -1/10th standard dose. We estimate that bleeding events in STEMI patients given reduced dose intra-coronary alteplase with radial artery access will be uncommon following 10 -20mg of alteplase ($\geq 1/1,000$ to $<1/100$).

1.3.4 Surrogate markers for bleeding

Fibrinogen: Thrombolytic therapy increases plasmin activity leading to both fibrin and fibrinogen breakdown. The degree of fibrinogen depletion correlates with the drop in fibrinogen (Fg) levels and rise in fibrinogen breakdown products (FgDPs) and is a likely marker of bleeding risk. Fibrinogenolysis is most marked with systemic thrombolysis (Streptokinase > alteplase/reteplase > tenecteplase) (3). Fibrinogen concentration represents a circulating marker of thrombolysis and bleeding risk (e.g. TIMI10A trial (26,43,53)). Since bleeding events are relatively uncommon (<5% incidence usually) fibrinogen concentration represents a surrogate marker of coagulation and bleeding risk which we propose to adopt in this trial. Typically, a 50% reduction in circulating fibrinogen concentration may occur within 3 hours of administration of standard systemic dose alteplase with almost complete recovery at 24 hours and rebound high levels at 48 hours post-treatment (26; Dr Campbell Tait, personal communication). The degree of systemic fibrinolytic activation may correlate with the fall in circulating plasminogen concentrations. D-dimer, a measure of Fibrin degradation, is markedly increased 90 minutes after thrombolysis with alteplase and TNK, and falls to near baseline concentrations by 24 h.

Fibrinolytic therapy also causes factor V activation and subsequent inactivation and depletion. The early activation within the 1st hour may contribute to unsuccessful thrombolysis or early re-occlusion. In contrast later ≥ 2 h loss of factor V may correlate with bleeding risk. Fibrinolysis may also lead to activation of coagulation via the kallikrein-Factor XII axis. This is evidenced by increased evidence of thrombin generation (and its byproduct Prothrombin F1+2) 30-90 minutes after fibrinolytic therapy. The procoagulant effect of thrombolysis may correlate with ineffective thrombolysis or early re-occlusion, but these deleterious pro-coagulant effects can be counteracted by anti-coagulation with e.g. heparin. With (or without) heparin therapy F1+2 normalises between 8-24 h after fibrinolytic drug administration. Thrombin generation (TG: a global measure of coagulation potential) could on the one hand correlate with bleeding risk, or on the other hand correlate with early risk of arterial re-occlusion, however, there is a paucity of information in the literature on thrombin generation in acute coronary syndromes or following thrombolysis.

1.4 Study rationale - hypothesis

Rationale: Despite numerous interventions, there remains a need to develop new ways to prevent MVO. We aim to select patients with persistent ST elevation on the ECG and an occluded artery and/or heavy thrombus burden at initial angiography. These characteristics are causally linked to MVO (4-26). Patients with a thrombotic culprit coronary artery have reduced myocardial perfusion compared to those without (59) and the presence of coronary thrombus is an independent predictor of adverse ischaemic outcomes post-MI (59,60). Intraluminal thrombus, as revealed by intra-vascular imaging with optical coherence tomography (OCT) in STEMI patients, has shown that thrombus commonly persists in the culprit coronary artery, including within the stent post-implantation (61), even when the thrombus is invisible with conventional angiography. The amount of persistent thrombus predicted the likelihood of persistent ST segment elevation, a marker of microvascular injury, and impaired perfusion (59,60). Therefore, coronary thrombus represents a therapeutic target in primary PCI.

Randomised controlled clinical trials of novel therapeutic approaches designed to reduce the extent and severity of infarction, including novel cardioprotective interventions such as intravenous beta-blocker therapy before reperfusion (EARLY BAMI; 62), intravenous inhibitors of mitochondria-mediated reperfusion injury (i.e. cyclosporine (CIRCUS; 63), TRO40303 (MITOCARE; 64)), and post-conditioning (DANAMI-3-POST; 65), deferred stenting (DANAMI-3-DEFER; 66), and intra-coronary vasodilator therapy (REFLOW; 67), and have not provided evidence of benefit. Only the Phase 2 METOCARD-CNIC trial (NCT02342522; 68) of intravenous beta-blocker therapy disclosed an improvement in the extent and severity of infarct characteristics, as revealed by MRI (68). However, the study population in this trial was restricted to patients with anterior STEMI, and given the results of EARLY BAMI (62), further studies are warranted. None of these interventions specifically targeted microvascular reperfusion. At the outset of the current trial, MVO is a common, adverse complication of acute STEMI for which there is no known treatment, and MVO remains a clinical problem of unmet need.

The pathophysiology of MVO and microvascular thrombosis has been elucidated in MRI studies of reperfused MI in swine (6, 57). We demonstrated that late MVO corresponds closely with infarct zone haemorrhage as revealed by T2*-weighted MRI and pathology (57). Our observations were validated by Robbers *et al* (58) who showed that in swine 7 days post-MI, when late MVO and haemorrhage correspond (which is usually the case), there is severe capillary loss and disruption coupled with thrombosis and inflammation. They concluded that following reperfusion, acute inflammation and microvessel thrombosis result in degradation of endothelial integrity and capillary breakdown. Very interestingly, their histology also demonstrated diffuse microvascular thrombosis within the area of late gadolinium enhancement surrounding the haemorrhagic core which explains reduced perfusion (or wash-

during first pass of gadolinium contrast MRI) within the ischaemic area-at-risk. This observation points to the therapeutic potential of local thrombolysis within the culprit artery circulation.

Our study addresses the question of whether a pharmacological strategy involving reduced dose alteplase given early during the primary PCI procedure will both *prevent* and *treat* distal microvascular thrombosis and MVO and, subsequently, reduce infarct size. Current evidence around the potential safety and efficacy of reduced dose fibrinolysis in primary PCI is limited. These limitations set-the-scene and support the rationale for our clinical trial:

Full systemic dose intravenous fibrinolysis to facilitate primary PCI is potentially harmful and increases the risk of off-target bleeding complications; therefore, we will use reduced-dose fibrinolysis. We will directly infuse alteplase into the culprit artery to achieve effective and sustained local plasma concentrations and much lower systemic concentrations of unbound drug. It is anticipated that bleeding rates may be low; therefore, we will measure fibrinogen in all patients. Fibrinogen and other haemostasis parameters will serve as a surrogate measure of bleeding (and safety). In line with contemporary practice, we advise that patients have radial artery access whenever possible.

Previous trials have used streptokinase (non-fibrin specific and immunogenic); we will use the fibrin-specific non-immunogenic second generation thrombolytic, alteplase. The only previous trial (27) involved thrombolysis at the end of primary PCI (when microvascular thrombosis may already be established after reperfusion); the efficacy of thrombolysis may be greatest when thrombus is most abundant at the beginning of primary PCI; persistent residual fibrin strands adherent within the culprit territory will be selectively targeted by fibrinolytic therapy during primary PCI; thrombus which forms during the primary PCI procedure could be treated by the sustained 'deep tissue' thrombolytic effects of locally administered intra-coronary alteplase; in terms of ease-of-use and feasibility, there may be advantages to giving alteplase as a single dose.

T-TIME is a Phase II evaluation of two reduced doses of alteplase, delivered locally, compared to placebo in STEMI patients receiving PCI in a double-blind, randomised, parallel group, placebo-controlled dose-ranging clinical trial. We believe our strategy with intra-coronary fibrinolysis complements other therapeutic approaches which are currently being tested (e.g. REDUCE-MVI: NCT02422888; ERIC-PPCI: NCT02342522). Should our trial demonstrate EFFICACY then a future trial might involve a factorial design with placebo, alteplase and any other intervention that might also be shown to be effective in the intervening time in order to test the comparative EFFICACY of each (alone or in combination) on surrogate and/or clinical outcomes.

Rationale for 'late' MVO as primary outcome, as revealed by contrast-enhanced MRI 2 -7 days post-MI: In STEMI patients treated by primary PCI, microvascular injury

revealed by the ECG and/or coronary angiography, is dynamic and the measurement accuracy of these methods is influenced by a number of factors, including timing, operator, reproducibility and measurement sensitivity.

Cardiac MRI performed 2 days post-MI discloses persistent microvascular injury (MVO) within the infarct core, as revealed by a central dark zone within a bright area of late gadolinium enhancement (2-4, 69-81). MVO imaged less than 2 days post-MI may diminish or resolve which is why the 2 day time-point is adopted as the earliest point for 'persistent' MVO (82). However observations from the BHF MR-MI study (83-85;NCT02072850) indicate that the amount of MVO is reasonably stable between days 2 – 10 post-STEMI however, MVO diminishes markedly between days 10 – 30. Given this and the fact that some patients may have to transfer between hospitals (invasive centre to base regional hospital) and since MRI on day 2 may not be feasible, the MRI scan can also be obtained at a later point; during the in-patient stay, at the time of hospital discharge or shortly after discharge, as appropriate, up to a maximum of 7 days post-MI. Persistent MVO is pathologically linked to non-viable myocardium and prognostically important. For this reason, MVO that persists on day 2 -7 detected with late gadolinium enhancement imaging is adopted as a clinically important surrogate outcome defined as 'late MVO'. Mechanistically, since intra-coronary alteplase targets fibrin-rich clot and microthrombi, which are causally implicated in MVO (5-10), a reduction in the incidence (i.e. prevention) and extent (i.e. treatment) of MVO would be directly linked to the mechanism of action of alteplase (2-29) and represent a clinically-important treatment effect (1,3,4).

Cardiac MRI is an established method for the assessment of heart function and injury post-MI for clinical (1,4,80,81) and research (18,20,76-79). MRI has greater measurement accuracy than echocardiography, and since MRI does not involve ionising radiation it can be safely repeated (unlike nuclear imaging) (79-80). Not only does MRI disclose MVO, but this abnormality is measured simultaneously with indices of left ventricular function and volumes, infarct size and other infarct characteristics such as area-at-risk and haemorrhage (69-89).

MVO may be imaged by MRI during myocardial 'first pass' of gadolinium contrast (i.e. a failure of contrast to 'wash-in'), or subsequently early (1 minute) or late (10-15 minutes) after gadolinium administration. As an extracellular contrast agent, gadolinium diffuses from capillaries into the interstitial space. Because of this diffusion, the extent of the wash-in deficit revealed by first pass MRI diminishes over time as revealed by early and late gadolinium enhancement (2, 69). The pathophysiological basis (57) and clinical significance of MVO revealed by dynamic first pass, or subsequently by late gadolinium enhancement, differs, and some longitudinal studies (2,3,72-75) have found that MVO which persists on late gadolinium enhancement is a stronger predictor of left ventricular outcomes.

Since intra-coronary alteplase during primary PCI should theoretically reduce microvascular thrombosis and improve perfusion, a reduction in the extent of the myocardial perfusion deficit within the area-at-risk would be a mechanistically relevant treatment effect (90-94). Adenosine stress MRI might enhance differences in perfusion between treatment groups. However, there are some theoretical limitations with the rationale for vasodilator adenosine stress CMR since PCI will have treated the culprit lesion, vasodilator effects may be inadequate and/or variable early post-STEMI, and a 3D rather than 2D imaging approach would be needed to calculate ischaemic burden. In practical terms, a protocol involving stress MRI including all other relevant scans (e.g. T1, T2, T2* maps) would be long (> 1 hour) and more difficult for a STEMI patient to tolerate, and a truncated scan without late gadolinium enhancement would lack the information essential for the primary outcome (late MVO). Therefore, our rationale is not to adopt stress MRI in this study. T1 mapping will be adopted instead of T2-weighted CMR since the area-at-risk and infarct characterization with T1 mapping is similar (pilot data, BHF MR-MI study NCT02072850; 83-85).

In terms of feasibility, although there are some contra-indications to MRI (e.g. permanent pacemaker, severe claustrophobia), MRI is widely performed in STEMI patients for clinical and research purposes (69-87) including in our hospitals (83-87,89) (see pilot data Section 6.7).

Certain patient characteristics may associate with the pathophysiology of MVO and myocardial haemorrhage, as illustrated in the BHF MR-MI study (NCT02072850). Age is associated with vascular risk and bleeding in relation to fibrinolysis. Cigarette smoking is associated with an accelerated vascular risk. The efficacy of the study intervention may associate with presenting characteristics such as the duration of ischaemia, anti-thrombotic therapy prior to reperfusion, and the patency of the culprit coronary artery at initial angiography. We anticipate undertaking pre-specified analyses of the primary and secondary outcomes in relation to these clinical characteristics. The analyses will be established in a statistical analysis plan before the end of the study.

Rationale for coagulation test: Initial baseline blood samples for coagulation will be obtained once verbal consent has been obtained, immediately after initial reperfusion (and before study drug administration) and then again, if feasible at, 2 hrs (± 1) and 24 (± 12) hrs afterwards. All of the samples will be sent to the hospital laboratory on each site for centrifugation, aliquotting and storage. Samples will be sent in batches to the McEwen Building, Glasgow Royal Infirmary. In the pre-specified SAFETY ANALYSIS (to be finalised with the Sponsor and IDMC), the coagulation results for the first 10% of the study participants will be made available to the IDMC in order to consider the unwanted systemic fibrinolytic effects (if any). This analysis will be coordinated by the NHS Greater Glasgow and Clyde Haemostasis Core Laboratory (Dr Campbell Tait) once the centrifuged plasma samples have been received from participating hospitals and analysed. The committee may also wish

to consider the coagulation results in any patients who may have experienced a bleeding complication.

Specific rationale for coagulation parameters as measures of safety (26,43,53):

1. Fibrinogen – depletion following fibrinolysis may correlate with degree of systemic fibrinogenolysis and bleeding risk.
2. Plasminogen activity – depletion following thrombolysis correlates with systemic fibrinolysis and may correlate with bleeding risk.
3. D-dimer – a specific measure of fibrinolysis (not fibrinogenolysis). D-dimer concentrations should correlate with amount of clot lysis therefore may be a measure of residual clot burden following aspiration (assuming residual clot is lysed). A minimal rise in D-dimer could therefore infer either: 1) there was minimal residual down-stream clot to be lysed or 2) any residual clot was not well lysed. D-dimer concentrations have the potential to correlate with efficacy and outcome.
4. Prothrombin F1+2 – a measure of thrombin activation, and correlates with the (undesired) procoagulant effect of thrombolysis. F1+2 will be depressed by anti-coagulants administered before and during PCI. Prothrombin F1+2 concentrations may correlate with the dose of alteplase and placebo and potentially could correlate with adverse outcome (i.e. a mechanistic and safety measure).
5. Tissue plasminogen activator (tPA)

Rationale for the coronary vascular function

We will measure prognostically validated angiographic indices of coronary artery blood flow (TIMI coronary flow grade (95)), microvascular function (TIMI perfusion grade (96), TIMI frame count (96-98)) and TIMI thrombus grade and intra-procedure thrombotic events ((IPTE) 99). These parameters will provide information on coronary vascular function and therefore on the mechanisms of potential efficacy of the study drugs.

Rationale for histopathology of thrombus: The efficacy of alteplase may be related to the % fibrin content in formed coronary thrombus (8,11,100). We wish to measure fibrin content using histopathology staining of fibrin within thrombus aspirates using central core laboratory methods (Dr Sylvia Wright, Consultant Pathologist, Dept. Pathology, Queen Elizabeth University Hospital, Glasgow). We will then correlate fibrin content with the primary and secondary endpoints for efficacy.

Rationale for treatment strategy: Our proposal exploits current knowledge with several novel approaches: 1) mode of alteplase delivery - slow local infusion (in line with the approach with intra-arterial thrombolytic infusion in acute stroke); 2) dose - very low dose (1/10th) vs. low

dose (1/5th) vs. placebo; and 3) optimal timing –after mechanical reperfusion but before stenting to lyse persistent coronary artery thrombus, attenuate distal embolisation and prevent/reduce MVO particularly as the deep tissue half life of alteplase is up to 40 minutes, thrombolysis may continue after stenting and post-dilatation to treat any persistent formed thrombus or new microthrombi; 4) MECHANISMS - to be studied invasively (angiography) and non-invasively (ECG, MRI). Therefore, alteplase or placebo administration will take place within standard care by happening after reperfusion but before stenting during PCI. Alteplase is therefore intended to complement the standard of care procedure which will not change.

Rationale for measurement of thrombus burden at the end of PCI with optical coherence tomography (OCT):

OCT – to be performed before and after study drug administration and at the end of PCI

OCT is a wire-based form of imaging that provides clear and detailed resolution of coronary artery thrombus. OCT is a light based form of intra-vascular imaging catheter which is straightforward to use, quickly acquired (< 5 seconds pullback) and provides very high quality images (spatial resolution of 10 - 20 μ m). OCT is the gold standard method of intra-coronary imaging, especially for assessment of thrombus in randomized trials in STEMI (101-104).

OCT has been used to measure the amount of thrombus in therapeutic trials of anti-thrombotic interventions (101-104). The first COCTAIL study was a placebo-controlled trial of intra-coronary abciximab (an anti-platelet drug) in patients with STEMI secondary to coronary thrombosis (101). There were 25 patients in each group and the mean percentage change in thrombus score was 33.8% in the abciximab-treated patients and 3.9% in the placebo-treated patients (p=0.002).

Based on the data reported by Di Giorgio et al (102) we plan to use OCT in 90 subjects in line with previous studies (i.e. COCTAIL) in sites interested in participating in the sub-study. The outcome measurement will be mean relative thrombus area at the end of the primary PCI procedure. The OCT images will be analysed in a blinded manner post-procedure using standardised core laboratory methods.

Rationale for measurement of coronary physiology parameters (the index of microvascular resistance (IMR) and coronary flow reserve (CFR)):

Physiological parameters of coronary function can be measured invasively using a standard diagnostic coronary guidewire. The index of microvascular resistance (IMR) is a guidewire-based test of microvascular resistance (105,106) and coronary flow reserve (CFR) is a test of coronary reactivity in response to vasodilator therapy (107-109).

IMR is defined as the distal coronary pressure multiplied by the mean transit time of three sequential manual bolus injections of saline (3 ml) at room temperature during maximal

coronary hyperemia, measured simultaneously (mmHg x s, or units) (105,106). Coronary flow reserve is defined as the mean transit time at rest divided by the mean transit time during hyperemia (107-109). A pressure- and temperature-sensitive coronary guidewire (St Jude Medical, Uppsala, Sweden) is used to measure IMR and CFR, which can be acquired as an adjunctive test in just a few minutes during primary PCI (110-112).

IMR is independently associated with left ventricular function (113), infarct pathology (114-116), and health outcomes post-STEMI (110,117). In a pooled analysis of 253 patients with acute STEMI followed for a median of 2.8 years, Fearon *et al* (117) found that an IMR >40 was a multivariable associated of all-cause death and heart failure. The prognostic importance of CFR in acute STEMI is less clear.

In the British Heart Foundation MR-MI study recently conducted in Glasgow in 324 patients with acute STEMI (110), we found that 1) IMR can be routinely measured in the culprit coronary artery at the end of emergency PCI; 2) IMR is associated with microvascular obstruction and myocardial hemorrhage; 3) compared with coronary flow reserve (CFR, another index of coronary function), IMR reflects more severe vascular damage, as reflected by myocardial hemorrhage, persistent ST-segment elevation, and Killip heart failure classification; 4) compared with IMR, CFR was discriminative of microvascular obstruction in patients with less severe myocardial injury, as reflected by the absence of myocardial hemorrhage, whereas IMR was not discriminative in this group; 5) IMR and CFR were associated with pro-inflammatory cytokines, as revealed by associations with log IL-6, and NT-proBNP; 6) IMR and CFR were associated with changes in left ventricular volume; whereas only IMR was a multivariable associate of adverse health outcome events during longer term follow-up (similar to persistent ST-segment elevation), unlike the duration of symptoms, CFR and angiographic parameters.

Cuculi *et al* recently reported that CFR was modifiable when assessed repeatedly within 24 hours of reperfusion (118), and the change in CFR was associated with infarct characteristics. IMR tended to be associated with microvascular obstruction, potentially reflecting the variability in IMR and the limited sample size. More recently, De Maria *et al* (119) observed that IMR remained persistently elevated in one third of patients at the end of primary PCI, and failure of IMR to improve by the end of the primary PCI procedure was multivariably associated with the extent of jeopardized myocardium, the amount of coronary thrombus and stent volume. These observations suggest that both CFR and IMR have potential to serve as an immediate biomarker of the efficacy of reperfusion in patients with acute STEMI. CFR may reflect changes in coronary function in patients with less severe infarction.

CFR and IMR may also reflect the effects of novel therapeutic interventions targeted to improve microvascular perfusion leading in turn to a reduction in coronary microvascular resistance. There is some evidence IMR is responsive to the effects of treatments known to

have favourable cardiovascular effects, including vasodilators (120), and anti-ischaemic (121) therapies. IMR increases following pre-dilatation before stenting vs. direct (primary) stenting without pre-dilatation (122). In patients with acute myocardial infarction, IMR is also a pre-specified outcome in on-going clinical trials of vasodilator therapy (123) and ticagrelor therapy (an anti-platelet drug with adenosine like-effects) (124, 125).

IMR and CFR will be measured before administration of study therapy and again at the end of the PCI procedure, using standard methods (110-113). Absolute coronary blood flow is related to IMR and is also measured by thermodilution. Based on data obtained in the BHF MR-MI study, where the tests of coronary function became adopted into routine care in the Golden Jubilee hospital (110,112), the coronary physiology sub-study may be performed in any of the participating sites by clinicians with experience of IMR measurement. The sample size is 256 subjects. The pre-specified outcome will be the between-group difference in IMR at the end of the PCI procedure. The physiology data will be analysed in a blinded manner off-line using standardised core laboratory methods.

Rationale for operator feasibility questionnaire: We are interested in capturing information on the feasibility of study therapy administration in clinical practice, including any problems with study drug administration, potential or actual drug errors, and overall safety. This information will be important in order to assess transferability of the intervention into the NHS in routine clinical practice. Information on these parameters will be evaluated to inform the design of any potential future Phase III trial.

1.5 Relevance to the NHS

Our study is intended to be applicable to clinical practice in the NHS and will provide developmental data which would support a large multicentre trial powered to detect treatment effects on 'hard' endpoints, such as mortality. Our study has the potential to contribute significantly to the scientific understanding and broader knowledge of the mechanisms and treatment of MVO which are currently very poorly understood. Since MVO and infarct size are major drivers of future heart failure, substantial health gain is plausible if our hypothesis proves correct. Our Phase II trial will robustly inform the design of a future multicentre Phase III trial based on health outcomes and cost-effectiveness which will facilitate adoption and impact in the NHS. The intervention fits well with the Department of Health's established plan of widespread adoption of primary PCI in the NHS (120,121).

2. STUDY OBJECTIVES

T-TIME is a double-blind, randomised, parallel group, placebo-controlled clinical trial designed to examine the efficacy and safety of reduced dose intra-coronary alteplase in STEMI patients receiving primary PCI. The primary objective is to determine the lowest effective dose of alteplase in reducing MVO. The results of T-TIME will inform the design of a larger definitive trial.

2.1 Primary outcome

The amount of MVO (% of left ventricular mass) revealed by late gadolinium contrast-enhanced MRI 10 – 15 minutes after contrast administration on an MRI scan performed 2 -7 days post-MI.

2.2 Secondary outcomes

A range of secondary outcome parameters will be measured and compared between groups, to further explore the efficacy of treatment and mechanisms involved. Primary and secondary outcomes will be analysed and submitted for publication as soon as possible after the 12 week assessments have been completed.

2.2.1 ACUTE

ANGIOGRAM

TIMI Coronary Flow Grade at the end of PCI,
TIMI Angiographic Blush Grade at end of PCI
TIMI Frame Count at end of PCI
TIMI thrombus grade at end of PCI

ECG

% ST segment resolution on the 12- lead ECG (pre- vs. 60 min post-reperfusion with primary PCI).

SAFETY

Acute cerebral (stroke) and systemic (GI, peripheral) bleeding (if any) with alteplase.
Coagulation (fibrinogen concentration; activated clotting time

2.2.2 EARLY (Day 2 -7)

MRI

Late MVO (presence / absence) 10-15 minutes after contrast administration

Infarct size

Myocardial salvage index (infarct size/area-at-risk)

LV end-diastolic volume (LVEDV)

LV end-systolic volume (LVESV)

LV ejection fraction (LVEF)

Myocardial haemorrhage (presence/absence)

Myocardial haemorrhage extent (% of LV)

Troponin T

QUALITY OF LIFE

EQ5D-5L

SAFETY

Acute cerebral (stroke) and systemic (GI, peripheral) bleeding (if any) with alteplase.

2.2.3 12 (±2) WEEK FOLLOW-UP

MRI

Infarct size

Myocardial salvage index (final infarct size/initial area-at-risk)

LV end-diastolic volume (LVEDV)

LV end-systolic volume (LVESV)

LV ejection fraction (LVEF)

ECG

ECG for final infarct size

QUALITY OF LIFE

EQ5D-5L

2.3 Tertiary outcomes

A number of additional (tertiary) outcomes will be collected at all assessment points up to 12 months, and long term. Tertiary outcomes will be analysed and submitted for publication in stages, after full database lock (when all participants have completed the 12 month assessment). All tertiary outcome data will be collected blind to randomised treatment allocation.

2.3.1 ACUTE

ANGIOGRAM

Intra-procedural changes in TIMI Coronary Flow Grade

Intra-procedural changes TIMI Blush Grade

Intra-procedural changes TIMI Frame Count

Intra-procedural changes in TIMI Thrombus Grade

Thrombus area (end of PCI and intra-procedural changes)

Intra-procedural thrombotic events

Intra-procedural thrombotic events

ECG

ST elevation score at 60 min post-reperfusion;

Cardiologist feasibility questionnaire

2.3.2 EARLY (Day 2 -7)

MRI

First pass MVO extent (% of LV)

Early MVO extent (% of LV) on 1 min post-gadolinium contrast enhanced MRI, adjusted for area-at-risk at baseline

LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness mid-diastole)

LV diastolic myocardial wall thickness to volume

LV sphericity index at end diastole (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter),

LV sphericity index at end-systole (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter),

Myocardial strain

Myocardial perfusion in the infarct zone

Myocardial perfusion in the remote zone

Infarct zone perfusion indexed to remote zone perfusion

LV wall motion

Myocardial haemorrhage at 3 months

Extracellular volume in the infarct zone

Extracellular volume in the infarct core

Extracellular volume in the remote zone

BIOCHEMISTRY

Creatinine (standard of care blood chemistry)

HAEMATOLOGY

Haemoglobin (standard of care blood results)

HISTOPATHOLOGY (SUB-STUDY)

Fibrin histopathology in thrombus aspirate

CORONARY PHYSIOLOGY (SUB-STUDY)

IMR

CFR

OPTICAL COHERENCE TOMOGRAPHY (SUB-STUDY)

Thrombus area

2.3.3 12 (\pm 2) WEEK FOLLOW-UP

MRI

First pass MVO extent (% of LV)

Early MVO extent (% of LV) on 1 min post-gadolinium contrast enhanced MRI, adjusted for area-at-risk at baseline

LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness mid-diastole)

LV diastolic myocardial wall thickness to volume

LV sphericity index at end diastole (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter)

LV sphericity index at end-systole (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter)

Myocardial strain

LV wall motion

Myocardial haemorrhage

Myocardial perfusion in the infarct zone

Myocardial perfusion in the remote zone

Infarct zone perfusion indexed to remote zone perfusion

ECG

Surrogate ECG measures of infarct size - Anderson ST Acuteness score and Selvester QRS score.

Acuteness of the ECG changes - Anderson Wilkins score.

2.3.4 LONG TERM FOLLOW-UP

Subsequent contacts will be by telephone, or by letter or clinic review as clinically appropriate. In the longer term, funding permitting, additional follow-up of health outcomes will be assessed by electronic case record linkage using the NHS number (England) and Community Health Index (CHI) number in Scotland with a minimum longer term follow-up of 3 years (average follow-up ~4.5 years).

QUALITY OF LIFE

EQ5D-5L at 52 (± 4) weeks and 104 (± 4) weeks

HEALTH OUTCOMES

Health outcomes (death, re-hospitalisation for cardiovascular events including for recurrent MI, heart failure, stroke/TIA, and acute bleeds) will be assessed at 52 (± 4) and 104 (± 4) weeks, with a final check for all participants at their final assessment this being defined as when the final randomised participant has completed 52 weeks follow-up.

3. STUDY DESIGN

T-TIME is a double-blind, randomised, parallel group, dose-ranging placebo-controlled clinical trial. This study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, 2006) and The Medicines for Human Use (Clinical Trials) Regulations, 2004 SI 2004:1031 (as amended). All investigators and key trial personnel will complete biennial GCP training.

3.1 Study population

The study will recruit 618 patients with STEMI referred to the participating study centres for primary PCI.

The standard of care is primary PCI. Therefore, following transfer to the cardiac catheter laboratory, male and female patients aged ≥ 18 years with acute (≤ 6 hrs) ST elevation myocardial infarction who are undergoing primary PCI will be evaluated for eligibility. The routine management of these patients will not be affected.

3.2 Setting

The study procedures initially take place in the cardiac catheterisation laboratory which is where patients with acute STEMI are treated by primary PCI. Screening, witnessed verbal informed consent, study drug administration and acute assessments of efficacy will take place during primary PCI in the cardiac catheterisation laboratory. The patient will then be transferred to the coronary care unit in the usual way.

3.3 Inclusion criteria

The decision to recruit patients will be made during the standard care procedure. An ECG is usually sent electronically to the Coronary Care Unit by the Ambulance Service or First Medical Responder (e.g. A&E staff). Clinical criteria will be evaluated when the patient arrives in the catheter laboratory.

STEMI patients will be identified based on the presence of characteristics associated with an increased risk of no-reflow/MVO, including all of the following: persistent ST elevation, heavy thrombus burden, and an occluded culprit artery.

CLINICAL

- Male ≥ 18 years of age; females ≥ 18 years of not of child bearing potential (defined as women who are post-menopausal or permanently sterilised (e.g. hysterectomy, tubal occlusion, bilateral salpingectomy))
- Acute myocardial infarction (symptom onset ≤ 6 hours) with persistent ST segment elevation or new left bundle branch block.
- Radial artery access.

ANGIOGRAPHIC (Angiographic criteria will be reviewed after initial coronary angiography)

- Proximal-mid occlusion (TIMI flow grade 0/1) of a major coronary artery (i.e. the right, left anterior descending, intermediate or circumflex coronary artery). The reason for this criterion is to ensure patients with a clinically-meaningful (i.e. moderate to large) area-at-risk are included. Coronary collaterals may reduce the extent of infarction within the jeopardised area-at-risk, therefore, patients with functionally significant collaterals defined as partial or complete filling of the culprit artery (Rentrop grade 2 or 3, respectively) will not be included.

Or

- Impaired coronary flow (TIMI flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2+).

The rationale for including patients with impaired antegrade flow at initial angiography (TIMI flow grade 2) in the presence of significant thrombus is that myocardial perfusion is impaired and at risk of no-reflow, distal embolisation and microvascular obstruction. Intra-coronary thrombolysis specifically targets these problems. TIMI 2 flow grade in the absence of thrombus grade 2+ does not fulfil the eligibility criteria. Impaired filling of the culprit artery should be confirmed by the cardiologist on two orthogonal views of the angiogram. The definition of a TIMI grade 2+ thrombus means that the clot is angiographically obvious and at least small in size (grade 2 represents definite thrombus with greatest dimensions less than or equal to half the vessel diameter; grade 3 represents definite thrombus but with the greatest linear dimension greater than half but less than two vessel diameters; grade 4: thrombus present—large size: as in Grade 3 but with the largest dimension greater than or equal to two vessel diameters) (89).

Specific considerations:

Anti-thrombotic therapy will be in accordance with optimal standard care before and during primary PCI. This is expected to include unfractionated heparin (minimum 5000 IU) at the first medical contact combined with dual anti-platelet therapy with aspirin and either 600 mg of clopidogrel, 60 mg of prasugrel or 180 mg of ticagrelor in line with standard care.

On arrival in the catheter laboratory the ACT should be checked to confirm prior anti-coagulation (target ACS 250 s) and supplementary heparin should be given, as appropriate, if not done before arrival or the ACT is less than 250 s. During primary PCI, the ACT should be checked every 20 min to ensure therapeutic anti-coagulation (i.e. ACT 250 s) during the PCI procedure in line with optimal standard care. Intravenous heparin should be re-administered as needed according to the ACT.

Glycoprotein IIb/IIIa therapy

GpIIb/IIIa inhibitor therapy should be administered for 'bail-out' as per clinical guidelines (1). The indications for bail-out GpIIb/IIIa inhibitor therapy include angiographic evidence of massive thrombus, slow or no-reflow, or a thrombotic complication (1). The choice of gpIIb/IIIa inhibitor is as per local practice. The recommended dose for abciximab (Reopro) is 0.25 mg/kg given as an intravenous bolus, followed by a continuous intravenous infusion of 0.125 microgram/kg per min (to a maximum of 10 microgram/min) for 12 hours. The recommended dose for tirofiban (Aggrastat) is 25 microgram/kg given as a bolus followed by an intravenous infusion of 0.15 microgram/kg/min for up to 24 hours.

Other parenteral therapy for no-reflow

Adjunctive medical therapy may be given before and/or during the primary PCI procedure to prevent and/or treat no-reflow. Prior to the initiation of T-TIME, there was limited or no evidence that intra-coronary treatment with any agent had beneficial effects for no-reflow. In a study by Vijayalakshmi et al (126), compared with IC administration of heparinised saline (n=50 subjects), either IC verapamil (0.5 mg in 10 ml of heparinised saline; n=49) or IC adenosine (30 µg in 10 ml of heparinised saline; n=51) improved coronary flow and wall motion at days 1 and 30 after PCI for an ACS. However, a systematic review by Su et al (128) published in the Cochrane Library concluded there is insufficient evidence. More recently, in the REFLOW-STEMI trial, compared with standard primary PCI (control group; n=86), high-dose IC-adenosine (2 – 3 mg; n=82) and IC-sodium nitroprusside (0.5 mg in total; n=79) during primary PCI (with split dosing before and after stenting) did not reduce infarct size or MVO measured by CMR 1 – 4 days post PCI. In a per-protocol analysis, high-dose IC adenosine was associated with an increased infarct size, a reduction in LVEF and an increase in MACE at 6 months. In small studies, IC nitrate and IC nicordandil (Sadamatsu K et al (131)) have been associated with favourable improvements in TIMI Frame Count. Systemic therapy has also been pursued leading to favourable effects on surrogate outcomes with oral liraglutide (Chen WR et al (129)), a glucagon-like peptide-1 (GLP-1) given 30 min before primary PCI, and IV N-acetylcysteine (Pasupathy et al (130)) and but not consistently with IV metoprolol (Roolvink et al (132)).

Bivalirudin

If bivalirudin is used as the main anti-coagulant then intravenous heparin (70 – 100 U/kg) should still be administered initially to ensure immediate therapeutic anticoagulation and then the bivalirudin should be continued during and after the PCI at the standard dose until the patient has returned to the ward for up to 4 hours in line with contemporary practice.

Following primary PCI, in line with optimal standard care in each site, dual anti-platelet therapy should be maintained for at least 3 months (6 months for a drug eluting stent) with clopidogrel, ticagrelor or prasugrel or as per standard practice at study site. Therefore, during the critical early treatment period, anti-thrombotic therapy will be optimal and standardised in all patients.

There is no upper age limit for inclusion. Although the risk of bleeding after alteplase is higher in older persons, this risk is diminished by using reduced dose alteplase, thus permitting the potential efficacy of this intervention to be evaluated in patients of all ages.

3.4 Exclusion criteria

Clinical criteria that would exclude the patient from the trial will be evaluated by medical, research and nursing staff when the patient arrives in the catheter laboratory.

CORONARY

- Normal flow in the culprit coronary artery at initial angiography (TIMI grade 3)
- Functional coronary collateral supply (Rentrop grade 2/3) to the culprit artery
- Previous infarction in the culprit artery (known or suspected clinically, e.g. wall motion abnormality revealed by echocardiography)

CLINICAL

- Cardiogenic shock (Killip Class IV)
- Multivessel PCI intended before the day 2-7 MRI
- Previous infarction in the culprit artery (known or suspected clinically)
- Body weight estimated to be <60 kg
- Non-cardiac co-morbidity with expected survival <1 year
- Contra-indication to contrast-enhanced MRI
- Pacemaker

- Implantable defibrillator
- Known impaired renal function (eGFR<30ml/min)
- Significant bleeding disorder either at present or within the past 6 months
- Patients with current concomitant oral anticoagulant therapy (INR > 1.3), including apixaban, dabigatran, and rivaroxaban
- Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
- Known haemorrhagic diathesis
- Severe hypertension (BP >180/110 mmHg) not controlled by medical therapy
- Major surgery, biopsy of a parenchymal organ, or significant trauma within the past 3 months (this includes any trauma associated with the current AMI)
- Recent trauma to the head or cranium (<2 months)
- Prolonged cardiopulmonary resuscitation (>2 minutes) within the past 2 weeks
- Acute pericarditis and/or subacute bacterial endocarditis e.g. valve mass or vegetation revealed by echocardiography
- Acute pancreatitis
- Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis
- Active peptic ulceration
- Arterial aneurysm and known arterial/venous malformation
- Neoplasm with increased bleeding risk
- Any known history of haemorrhagic stroke or stroke of unknown origin
- Known history of ischaemic stroke or transient ischaemic attack in the preceding 6 months
- Dementia
- Hypersensitivity to gentamicin or natural rubber
- Incapacity or inability to provide informed consent
- Previous randomization to this study or participation in a study with an investigational drug or medical device within 90 days prior to randomisation
- Women of child-bearing potential (i.e. pre-menopause) or breast feeding.
- Requirement for immunosuppressive drug therapy at any time during the past 3 months; whether administered orally, subcutaneously, intramuscularly or intravenously. This would include corticosteroids (but not inhaled or topical), drugs used following transplantation (e.g. tacrolimus, cyclosporine), anti-metabolite therapies (e.g. mycophenolic acid (Myfortic), azathioprine, leflunomide (Arava)), and immunomodulators including biologics (e.g. adalimumab (HUMIRA), etanercept (Enbrel), aldesleukin), and DMARDS (cyclophosphamide, methotrexate, etc). Please note this list is not exhaustive and a requirement for other immunosuppressive drugs not listed would also exclude the patient.
- Active or prophylactic treatment with oral or parenteral antibiotic, antifungal or antiviral therapy to prevent or treat infection.
- Any anti-cancer treatment (excluding surgery as this is covered above) at any time during the past 3 months including chemotherapy, radiotherapy and treatment with biologics such as Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitors

(e.g. bevacizumab, pazopanib). This list is not exhaustive and sponsor or CI should be contacted for advice if required.

- Any significant concurrent or recent condition(s) not listed above that in the opinion of the treating clinician would pose an additional risk for the patient.

3.5 Identification of participants and consent

Consecutive STEMI patients will be screened on arrival at the hospital and will be considered eligible according to the presence of inclusion criteria and absence of exclusion criteria.

Screening will be performed by the cardiologist/nurse during and out-with office hours (i.e. 24/7) as appropriate. The decision as to whether a patient is eligible to be included in the study will be made and documented by the cardiologist.

Since enrolment will take place in the catheter laboratory informed consent will be obtained verbally. A standard approach to witnessed informed consent will be undertaken (3.5.1.1). Written consent will then be obtained after the patient has been moved to the Coronary Care Unit, as described in Section 3.5.1.2. Patients will be recruited to the sub-studies at the same time, and in the same manner as to the main study.

Screening log

The screening log will record consecutive patients but without any identifiable data. Each STEMI patient will be given a screening log number. Baseline characteristics and the reason for not being included according to the inclusion and exclusion criteria will be recorded in a screening log database, but no follow-up information will be recorded in this database. The reasons for not enrolling in the study or not receiving study drug therapy (e.g. presence of exclusion criteria, absence of inclusion criteria) will be prospectively recorded at the time of the procedure. As a derivative of the screening log, patients who are screened and who give informed consent will be recorded in the eCRF. Some patients who are initially eligible on clinical grounds but who subsequently become ineligible (e.g. on angiographic grounds) will not proceed in the study and will become screen failures. Rarely, some patients may be treatment allocation failures (e.g. for logistical reasons) but their data will still be recorded in the eCRF.

3.5.1 Summary of consent procedure

Because of the emergency nature of their acute illness, and the distress for the patient of having a heart attack, it would be inappropriate to delay treatment in order to obtain full informed consent in the standard way and therefore the procedures to be adopted are outlined below.

3.5.1.1 Informed consent in the catheter laboratory

Only patients who are sufficiently well to understand the information about the study (including the potential for benefit and known risks), as described by the attending cardiologist, would be eligible to participate. Patients who undergo a clinically-indicated emergency primary PCI and who are eligible to take part in the project will be invited to give verbal informed consent by the cardiologist responsible for the primary PCI. This discussion of the study will take place in the catheter laboratory in the presence of the catheter laboratory personnel, and will cover all of the points listed in the Short Patient Information Sheet. If the patient agrees to take part in the study then a form to this effect will be signed by the researcher. The decision to include a patient in the study must also be clearly documented by a study clinician in the medical notes and/or eCRF. The decision to include a patient in the study is the responsibility of the attending cardiologist.

3.5.1.2 Informed consent in the cardiology ward

After the primary PCI procedure is finished and the patient has returned to CCU he/she is then provided with a full Patient Information Sheet. Clinical staff will be available to discuss the study with the patient and his/her family and friends. The patient would then be invited to provide consent in writing on the full consent form. Full informed consent must be obtained within 24 hours of admission or prior to hospital transfer if this is earlier. When written consent is not possible e.g. the patient is unwell, then consent may be delayed and obtained when this becomes feasible. No further scheduled study assessments can be performed until full informed consent is obtained. The attending cardiologist must witness the written consent.

Our practice for recruiting acute STEMI patients in clinical trials is generally consistent with the processes in other hospitals in the UK and internationally.

Withdrawal criteria: A participant may withdraw from the study at any time. There are no specific withdrawal criteria, although clinicians can withdraw patients as appropriate and record the reasons. These patients would also be followed-up unless consent is withdrawn.

3.5.2 Recruitment plan

Project timetable and milestones:

RECRUITMENT RATE: on average, 2-3 patients per week per centre (8-12 per month) for the duration of the study (Consort flow diagram, Appendix 1). All patients will be recruited within 2 years of the start of recruitment (Gantt chart, Appendix 3).

PARTICIPANT DROP-OUT: Our sample size estimate allows for failure of randomised patients to undergo MRI (e.g. due to claustrophobia in randomised patients), problems with data acquisition and/or drop-out in up to 10% of participants, in line with our recent experience in clinical trials with MRI.

3.6 Study schedule

The study activity carried out in the first 24 hrs will take place during the initial hospitalisation. The day 2 -7 visit may be carried out during initial hospitalisation however subsequent visits will be on an outpatient basis.

Catheter laboratory

Primary PCI standard care: standard care for coronary reperfusion including optimal anti-thrombotic therapy including oral anti-platelet therapy and heparin (5000 IU or as per standard practice) at first medical contact if not previously administered. The target ACT is 250 s. The ACT should be checked at 20 minute intervals during the PCI procedure to ensure therapeutic anti-coagulation is maintained. Glycoprotein IIb/IIIa inhibitor therapy should be used with caution given the potential to promote bleeding in association with fibrinolytic therapy and according to clinical guidelines (1) for bail-out indications including angiographic evidence of massive thrombus, slow or no-reflow, or a thrombotic complication (1). The choice of gpIIb/IIIa inhibitor is as per local practice. The recommended dose for abciximab (Reopro) is 0.25 mg/kg intravenous bolus, followed by a continuous intravenous infusion of 0.125 microgram/kg/min (to a maximum of 10 microgram/min) for 12 hours. The recommended dose for tirofiban (Aggrastat) is 25 microgram/kg given as a bolus followed by an intravenous infusion of 0.15 microgram/kg/min for up to 24 hours and not less than 12 hours. Please see current Summary of Product Characteristics for further information on administration etc.

Intra-arterial nitrates and/or verapamil may be given routinely by some operators for one or more of the following reasons 1) to prevent vasospasm after insertion of the radial artery sheath, 2) to alleviate arterial hypertension, and 3) to prevent and/or treat no-reflow during the PCI procedure. Intra-coronary adenosine may be given for (3). Other therapies for the

prevention and treatment of no-reflow e.g. sodium nitroprusside, nicordandil, are rarely used in the UK.

Standard care checks

Vital sign observations (heart rate and rhythm, blood pressure) and check of activated clotting time (ACT)

Height and weight (measured as per standard care and required to plan MRI)

ECG

Blood samples at sheath insertion (before reperfusion)

Troponin T

Complete blood count and ACT

Blood chemistry

Eligibility checks

Evaluation of inclusion criteria and absence of exclusion criteria

Witnessed verbal informed consent.

Study procedures

As per standard care checks and,

Study blood samples (30 ml) for central laboratory check of baseline tests (coagulation, troponin T, biomarkers). NB Research blood tests at this timepoint are preferred but not compulsory

Thrombus aspiration (for histopathology)

Study consent and randomisation

Patient consent and randomisation. Treatment allocated using IVRS/IWRS system

Check ACT and administer unfractionated heparin as appropriate.

T0: Study drug administration (single infusion over 5 - 10 min) in catheter laboratory at start of PCI (after successful reperfusion (TIMI flow grade ≥ 2) but before stenting)

Vital sign observations (heart rate and rhythm, blood pressure)

ACT check at 20 min intervals during the PCI and administration of unfractionated heparin as needed

See Study Flow Chart

- ⇒ Aspiration thrombectomy may be performed for thrombus-containing lesions on a selective basis, as per contemporary guidelines. Balloon angioplasty may be performed to stabilise the plaque and prevent re-occlusion by use of a balloon sized

<1:1 with respect to the vessel diameter and inflated at low balloon inflation pressures in order to minimise thrombus embolisation and maintain successful coronary reperfusion (TIMI coronary flow grade ≥ 2).

- ⇒ The study pack is determined by the randomisation system with either the voice/telephone (IVRS) or web-based (IWRS) trials unit system
- ⇒ *Dissolve study drugs* (1 vial per 10 ml diluent into one syringe e.g. 20 ml)
- ⇒ *Intra-coronary infusion catheter (e.g. aspiration thrombectomy catheter or perfusion catheter)*: The catheter must be fully flushed with 0.9% sodium chloride immediately prior to administration of the study drug. The catheter should then be advanced on the coronary guidewire into the culprit coronary artery proximal to the lesion.
- ⇒ In the case of a RCA or LMS lesion, the guide catheter may be used for drug infusion (assuming there is good engagement of the guide catheter with the coronary ostium).
- ⇒ Infuse study drug (20 ml) – slow, manual infusion of up to 10 minutes but a minimum of 5 minutes (slow manual infusion rate = 2 ml / min) If a thrombectomy catheter is used to infuse the study drug, the catheter may be intermittently withdrawn on 1 – 2 occasions (e.g. for a 1 minute period) to facilitate antegrade blood flow.
- ⇒ Finally, flush the catheter with normal (0.9%) saline to ensure that all of the study drug has been completely administered

Detailed information on study drug preparation and administration is provided in the study 'IMP Management and Accountability Manual'

T 0 - 2 hrs: Acute efficacy measurements (catheter laboratory and CCU)

Vital sign observations (heart rate and rhythm, blood pressure)

ECG

Coronary angiographic parameters

- initial angiogram (before and after reperfusion): coronary flow and thrombus in the culprit artery

- final angiogram: coronary flow, thrombus, thrombotic events, frame count and blush in the culprit artery

Mean change in thrombus score will be determined by analysis of the angiographic images in a blinded manner using standardised core laboratory methods.

Adverse event assessment

Cardiologist feasibility questionnaire, to capture information on ease-of-study drug administration, compliance/completeness of study drug administration, complications, feedback

T 2 hrs± 1: Early efficacy and safety measurement (CCU)

Vital sign observations (heart rate and rhythm, blood pressure)

ECG 60 min after reperfusion with minimum ST segment elevation

Medical/Disease History

Drug therapy

Study blood sample (30 ml) for central laboratory coagulation test and biomarkers (e.g. troponin T) NB Research blood tests at this timepoint are preferred but not compulsory.

Adverse event assessment

T 24 hrs± 12: Early efficacy and safety measurement (CCU)

Written informed consent

Vital sign observations (heart rate and rhythm, blood pressure)

ECG

Study blood sample (30 ml) for central laboratory coagulation test and biomarkers (e.g. troponin T) NB Research blood tests at this timepoint are preferred but not compulsory.

Complete blood count

Blood chemistry

Concomitant medication review

Adverse event assessment

T 2-7 days: Early efficacy and safety measurement (CCU/Cardiology ward)

Vital sign observations (heart rate and rhythm, blood pressure)

ECG

Study blood samples (30 ml) for central laboratory test of NT-proBNP and troponin and coagulation markers

Contrast-enhanced cardiac MRI (Section 2.2; primary outcome)

EQ5D QoL assessment

Adverse event assessment

T 12 weeks \pm 2: Follow-up efficacy and safety measurement (hospital visit)

ECG

Study blood samples (20 ml) for central laboratory test of NT-proBNP and troponin

Contrast-enhanced MRI (non-stress)

QoL assessment (EQ5D)

Adverse event assessment

Secondary and tertiary MRI parameters (see Section 2.2)

T 12 weeks \pm 2, 52 weeks \pm 4, 104 weeks \pm 4 and final assessment as outpatient

Adverse event assessment by telephone initially or patient review as appropriate.

QoL assessment (EQ5D)

T 3 years / long-term follow-up

Electronic case record linkage for adverse events (rehospitalisation and/or death) based on the Community Health Index or NHS number; no patient contact. The longer term follow-up duration will be a minimum of 3 years for all participants (anticipated average follow-up will be 4.5 years) and will be dependent on the availability of funding. The long term follow-up analysis is not covered by the NIHR-EME grant.

3.7 Assessments and Procedures

All study assessments will be made blind to treatment group allocation and clinical outcomes. The data for each subject will be coded and de-identified and analysed in random order.

3.7.1 Primary outcome analysis

3.7.1.1 Imaging with MRI

Gadolinium contrast-enhanced MRI will be performed at 1.5 Tesla at two days, or as soon as possible up to a limit of 7 days, post-MI (primary outcome scan) and 12 weeks \pm 2 post-MI. MRI at 2 days is the established minimum time post-MI for late MVO (3,4,69-85). Since infarct remodelling is substantially complete by 12 weeks post-MI (72), with little if any further changes to be anticipated by 24 weeks, 12 weeks \pm 2 post-MI is adopted for the secondary outcomes.

MRI outcome definitions

Microvascular obstruction:

DAY 2 -7 MRI (Late MVO, primary outcome): Late MVO is defined as a central dark zone present on first-pass, early delayed enhancement imaging 1, 3, and 5 minutes post-contrast injection and persistent within an area of late gadolinium enhancement 10 min after contrast injection (3,4,69-85). The presence or absence of late MVO is relevant to the primary outcome, but the primary outcome is the amount of MVO (% of left ventricular mass). In line with other laboratories, an established analysis approach e.g. full width half maximum, will be used to delineate the border of MVO on MRI images according to the MRI SOP.

MVO will be imaged with MRI scans timed to acquire images during the 'first pass' of gadolinium contrast in the ventricular myocardium, or 'early' or 'late' after contrast administration (69). This form of MVO is closely linked with non-viable myocardium (72,73). MVO is classified as relevant (central dark zone with a sub-endocardial or intra-mural distribution) and non-relevant (dots or nil) and quantified as a percentage of total left ventricular mass, after adjustment for the initial area-at-risk, as revealed by T2-weighted MRI (71). 'Early MVO' is acquired up to 1 minute after gadolinium administration whereas late MVO, as defined above, persists 10 - 15 min after contrast administration (69). The extent of 'first pass' and 'early MVO' may be greater than late MVO (69), due to passive diffusion of gadolinium and/or collateral supply, and late MVO represents persistent capillary obstruction (2-4,69-74). Quantitative analysis of first pass perfusion MRI may reflect tissue blood flow (perfusion, ml/mg/min) within the infarct zone and indexed to the remote zone, in turn reflecting microvascular function. Myocardial perfusion within the infarct zone revealed by quantitative methods therefore has the potential to reflect the efficacy of reduced dose thrombolysis administered within the culprit coronary artery, as compared with placebo. First pass perfusion may under-estimate the true extent of MVO if less than full LV coverage is not acquired during imaging (69).

Of the three types of MVO, most prognostic studies have found that late MVO is the most tightly linked with adverse remodelling (72,83) and clinical outcomes (3,74,75).

Approximately half of the patients are expected not to have evidence of any form of MVO on late gadolinium enhancement MRI, however the amount of late MVO is preferred for the primary outcome. From a statistical point of view, MVO expressed as a continuous trait will be skewed and with many zero values recorded for patients without MVO (around half of the study participants), the MVO values will require some form of transformation (e.g. log transformation) and potentially a non-parametric method of analysis.

Late MVO is an independent predictor of heart failure and death and represents a patient focused clinically important outcome that is mechanistically relevant to the mode of action of alteplase. Microvascular obstruction has disclosed treatment effects in previous randomised

clinical trials (18,76,77,79). When interpreting the results of the trial, all data and results will be considered to form an overall strategic view relevant to the future Phase III trial.

Area-at-risk

The jeopardised area-at-risk on each axial image is defined as the percentage of left ventricular area delineated by the hyperintense zone on T2-weighted MRI according to the Standard Operating Procedure. Results from the BHF MR-MI study (NCT02072850) indicate that the area-at-risk revealed acutely by T1-mapping and T2-mapping are similar. The area-at-risk is also revealed by contrast-enhanced cine imaging (64).

Infarct size

The presence of acute infarction will be established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and late gadolinium enhancement imaging. In addition, supporting changes on the ECG and coronary angiogram were also required. Acute infarction will be considered present only if late gadolinium enhancement is confirmed on both the short and long axis acquisitions, including with repeated MRI with change in the phase-encode direction in order to assess for and exclude artefacts. The myocardial mass of late gadolinium (grams) will be quantified by a semi-automatic detection method established in the Standard Operating Procedure, in line with current standard practice in many MRI laboratories worldwide (74-78). Infarct regions with evidence of microvascular obstruction will be included within the infarct area.

Myocardial salvage

Myocardial salvage will be calculated by subtraction of percent infarct size from percent area-at-risk (64,78,83-85). The myocardial salvage index will be calculated by dividing the myocardial salvage area by the initial area-at-risk.

Haemorrhage on T2*-weighted CMR

Myocardial haemorrhage revealed by T2* weighted imaging is defined as a confluent dark zone with a mean signal intensity < 2 standard deviations of the mean signal intensity of the surrounding affected brighter area (57,58,83-85). Haemorrhage will be expressed as a percentage of total left ventricular mass.

First pass MVO

In an exploratory analysis, the extent of the first pass perfusion deficit at rest (i.e. first pass MVO) will be quantified in a summative manner to estimate the number of segments involved (90-94). This protocol will not involve adenosine stress MRI but rest perfusion will be assessed.

Extracellular volume

Myocardial extracellular volume in regions of interest will be estimated using T1 mapping before and 15 min after gadolinium contrast administration.

Left ventricular remodelling

Changes in left ventricular dimensions and volumes will be assessed at 3 months and changes from baseline will be determined. Adverse remodeling is defined as an increase in LV end-diastolic volume $\geq 20\%$ from baseline (85). The LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness in mid-diastole), and LV sphericity index (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter) at end diastole and at end-systole will be assessed. The LV sphericity index is calculated by dividing the maximal longitudinal LV diameter (ie.mitral valve tip to LV apex) by the maximum short-axis diameter, at end-diastole and end-systole, using the vertical long-axis cine MR image. An index approaching 1 indicates increased sphericity. In order to express left ventricular cavity remodelling in relation to the LV myocardial remodelling, the diastolic myocardial wall thickness to volume ratio will be quantified using the end-diastolic remote myocardial wall thickness and the indexed LVEDV values. The clinical significance of LV remodelling will be determined by associations with the occurrence of adverse cardiac events at 12 months.

MRI

The MRI scan will include contrast-enhanced steady-state free precession (CE-SSFP) MRI, T1 and T2* maps, strain encoded MRI, and contrast enhanced imaging including first pass perfusion at rest, microvascular obstruction and late gadolinium enhancement imaging 10 - 15 min after contrast administration at 1.5 Tesla on a Siemens or Philips scanner. Multivendor protocols are common in contemporary clinical trials with MRI (19,23). The scan parameters will be closely matched and the specific details of the protocol are detailed in a Standard Operating Procedure. Intravenous access will be needed for contrast infusion.

MRI protocol

MRI will be performed on 1.5 Tesla scanner with a standard (e.g. 8-element) phased array cardiac surface coil.

The MRI protocol includes free breathing localisers including sagittal, coronal and axial acquisitions, then T1-weighted MRI scans, 3 short axis scans of the base, mid and distal positions of the left ventricle and one long axis view where feasible (vertical long axis, horizontal long axis and/or 3 chamber view). The slice selection should ensure that the infarct area is included in at least one of these scans. Contrast-enhanced cine imaging with steady state free precession will enable assessment of the area-at-risk (64). In addition, a left ventricular short axis (SAX) stack will be obtained for T2* parametric maps. Next a stack of short axis slices for first pass gadolinium contrast perfusion MRI will be acquired to maximise LV volumetric coverage followed by early (1, 3 and 5 minute) and late (10 - 15 minutes) gadolinium enhancement MRI acquired with a short axis LV stack and at least one long axis view. The I.V. contrast agent is gadolinium (Gadovist®, Bayer) administered at 0.15 mmol/kg. The cine-MRI for LV mass and function will be collected during the interval between the 5 min scan and late enhancement imaging. Post-contrast T1 mapping MRI will be repeated after the late gadolinium MRI scans, whenever feasible. A full blood count will be obtained before the scan in order to measure extracellular volume.

MRI scan imaging parameters

Cardiac mass and function will be assessed using steady-state free precession (SSFP) cine breath-hold sequences (with parallel imaging acceleration). The heart will be imaged in multiple parallel short-axis (SAX) planes 8-mm thick separated by 2-mm gaps, equating to approximately 10 slices and 30 cardiac phases.

T1 imaging will be planned taking care to ensure the left ventricular outflow tract is excluded from the basal slice.

Quantification of myocardial T1 relaxation time

Myocardial longitudinal relaxation times (T1, ms) are influenced by tissue water content and pathology. A T1 mapping method will be used, e.g. MOLLI, as outlined in the MRI guideline. The time interval for T1 map acquisitions will be recorded after the gadolinium contrast bolus.

Quantification of myocardial T2* relaxation time

A T2* mapping method will be used as outlined in the MRI guideline.

Contrast-enhanced MRI

An automated pump injector will be used for I.V. injection of 0.15 mmol/kg of gadobutrol (Gadovist®, Bayer Healthcare) with an automated pump injector (e.g. Medrad). First-pass perfusion at rest for 'wash-in' MVO quantification will be performed with a fast low-angle shot (FLASH) sequence run simultaneously with the contrast injection. An inversion recovery-prepared T1-weighted gradient echo pulse sequence will be used. Alternate heart beat acquisition will be run (unless HR<55) and the inversion time will be set according to a Look-Locker scout scan. Alternatively, a phase sensitive inversion recovery (PSIR) method will be applied to optimise image quality.

MRI of MVO

First-pass contrast-enhanced MRI will be performed with an appropriate pulse sequence. Typical imaging parameters could be: 8 left ventricular slices over 2 R-R intervals and no inter-slice gaps, field of view = 350 mm x 450 mm, flip angle = 15°, a repetition time = 3.7 ms, echo time = 1.0 ms, 7-fold acceleration, 11 training profiles, 1.5 mm x 1.5 mm x 10 mm voxel size (spatial resolution) (69). Early MVO will be defined on the MRI scan obtained 1 min after contrast administration.

LATE MVO and scar will be imaged 10-15 minutes after intravenous Gadovist® contrast administration using for example motion-corrected T1-weighted phase-sensitive inversion recovery radiofrequency pulse sequence (75-83). Phase-sensitive inversion recovery MRI techniques reduce variability associated with myocardial nulling which is required for late gadolinium enhancement imaging of infarct vs. unaffected myocardium (79). If a phase-sensitive protocol is not used, a modified Look-Locker inversion time scout will be performed prior to using an inversion recovery turbo gradient echo sequence. Phase swaps will be performed where appropriate to rule out artefact. A single shot technique or navigated late gadolinium enhancement MRI will be used as an option for poor breath holders. Since MVO is typically present within the central zone of infarction most often located in the mid-distal part of the ventricle, rather than the base or apex, late gadolinium imaging 10-15 min after contrast administration will start at the mid-papillary level and progress centrally towards the apex and base on alternating scans.

Typical imaging parameters A are included in the MRI guideline. MVO will be quantified from these images.

MRI Follow-Up: MR scans at 12 weeks ±2 follow up will involve a similar scan protocol.

MRI core laboratory image analysis

A clinical report should be issued for each MRI scan in line with local standards of clinical care. A de-identified electronic copy will be made available by secure web-upload to the core laboratory in Glasgow, or when this is not feasible, then by CD/DVD. The scan will include the patient study code and date of the scan. The MRI analyses will be conducted in line with local standard operating procedures (SOP).

MRI Core Laboratory (PI Berry, Radjenovic): Each MRI scan will be logged and assessed for quality (poor, acceptable, good) and feedback will be provided to the local site, as appropriate. The scans from all of the centres will be coded and de-identified and analysed blind to treatment group assignment and clinical outcomes. MRI scans will be prospectively analysed by cardiologists and radiographers with expertise in cardiac MRI. Left ventricular mass and function and infarct characteristics will be analysed by trained staff. All of these data will be reviewed and approved by the MRI cardiologist (Prof Berry) and quantitative analyses will be assured by Dr Radjenovic. Therefore, all scans will be reviewed by at least 2 trained observers. MRI measurements including LV dimensions, LVEF, myocardial strain, area-at-risk (T1-weighted MRI), infarct scar, haemorrhage, MVO, and for remodeling, the LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness in mid-diastole), and LV sphericity index (maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) / maximal short-axis diameter) at end diastole and end-systole will be prospectively analysed and recorded in a database. In order to express left ventricular cavity remodelling in relation to the LV myocardial remodelling, the diastolic myocardial wall thickness to volume ratio will be quantified using the end-diastolic remote myocardial wall thickness and the indexed LVEDV values. T1-weighted MRI will be used to delineate area-at-risk, myocardial salvage. Haemorrhage will be assessed with T2* MRI. T1, and T2* relaxation times measured in regions of interest (infarct core, infarct zone, remote zone) and extracellular volume will be mechanistic (tertiary) rather than secondary outcomes (see Section 2.2).

MRI analyses will be performed on dedicated workstations with customised software (e.g. QMASS, Medis, Leiden). The 'industry-standard' software enable semi-automated standardised thresholding and border-delineation of areas-of-interest (i.e. gadolinium hyperenhancement due to infarction and hypoenhancement due to MVO and/or haemorrhage). User-involvement will be retained to adjust endocardial/epicardial borders (e.g. exclude blood pool) and occasional image artefacts. This software is an essential part of a core laboratory approach. For paired measurements for secondary outcomes, the baseline and follow-up MRI scans will be analysed together (side-by-side). This approach will ensure standardised settings and measurements for the baseline and follow-up scans.

Analysis of first pass contrast MRI: Using the modified ACC/AHA 16-segment nomenclature, segmental first pass will be interpreted as normal or abnormal. Each segmental abnormality

will be scored on the basis of the transmural extent of the perfusion defect (0 = no defect, 1 = 1% to 50%, 2 = 51% to 100%). The apical cap (segment 17) will not be assessed because of the short-axis acquisition, so this segment will be treated as missing (i.e. 16 segment model). A perfusion defect will be deemed relevant only if it persists beyond peak myocardial enhancement. In an exploratory analysis, since non-transmural perfusion defects can be discriminated with high spatial resolution MRI, the myocardium will be divided into endocardial and epicardial segments thus resulting in 32 segments in total (91). The data will also be expressed as myocardial perfusion within the infarct zone, the remote zone and the infarct zone indexed to the remote zone. The quantitative analyses of myocardial perfusion will be directed by Dr Radjenovic.

Reproducibility of MVO: The day 2 MRI scans of 25 consecutive STEMI patients treated with primary PCI in the Golden Jubilee National Hospital (September-October 2012) were independently assessed by 2 experienced observers. The MRI scans were analysed for the presence and extent of late MVO. There was 100% agreement between the 2 observers for MVO (Cohen's Kappa statistic = 1). Twenty patients had MVO and the mean±SD extent of MVO in these patients were 2.9±3.4% and 3.2±3.6%, respectively. The 95% confidence intervals for systematic bias between the observers was -0.64% to 0.18% which indicates that there was no evidence of bias (p=0.26). The 95% limits of agreement were -2.2% to 1.7%.

Reproducibility of Area-At-Risk (n=8 STEMI patients). The 95% limits of agreement for AAR estimation by 2 independent observers were -12% and 15%, without evidence of bias (P=0.14) (57,79).

Quality assurance

There will be a continuous quality assurance process throughout the trial with feedback to sites on MRI scan quality. An NHS medical physicist from the lead site will assess and support the optimisation of MRI scan acquisition and image quality. An assessment of MRI data quality will be obtained from each site before enrolment of study participants, including of a T1 phantom for calibration, wherever possible.

3.7.2 Secondary outcome assessments

The secondary outcomes in this trial are pre-defined for treatment efficacy or mechanisms evaluations. The secondary outcomes for mechanisms are intended to inform the interpretation of the main results of the trial (primary outcome, secondary outcomes for efficacy). The mechanisms evaluations

(tertiary outcome) will be reported in full after the main results of the trial.

3.7.2.1 Electrocardiogram (ECG)

Acute ECG endpoints:

Summative ST elevation score on the 60 min ECG post-MI (122-124);

Surrogate ECG measures of infarct size include the Anderson ST Acuteness score (123) and the Selvester QRS score (124).

The acuteness of the ECG changes will be assessed from the Anderson Wilkins score (123). In the second ECG, the distribution of Q waves is of interest in respect of its relationship to any necrotic area of myocardium. Therefore, the ECG data could be correlated with the MRI and coronary angiographic data to undertake work on the topics described. The ECG Core Lab software will undertake infarct sizing using the method of Selvester (124) so that ECG estimates of infarct size can be compared with MRI estimates.

Although separate to the aims of the current study, the ECG and MRI data will represent a resource for future analyses. For example, little is known about the distribution of ST elevation in initial stages of an STEMI and how it relates to the injured myocardium and culprit artery. There are still no sensitive and specific criteria to detect an occlusion of the left circumflex artery from an ECG. These studies could enhance current knowledge on the diagnosis and management of acute MI. New knowledge could be incorporated into the Glasgow ECG Analysis Program, used widely in hospitals round the world, to enhance the diagnostic accuracy and clinical value of the automated ECG reports.

ECG CORE LAB (PI Macfarlane):

A 12 lead ECG will be performed according to standard methods. The ECG will be acquired by trained cardiology staff using standard ECG recorders available in the cardiology department. ECGs will be prospectively collected and uploaded to the e-CRF portal for electronic transfer to the core laboratory during the study.

The University of Glasgow ECG Core Lab (PI Prof Peter MacFarlane) has well established methods for processing of ECGs from various clinical trials and research studies. The lab is certified to ISO 9001: 2008 standards by a UKAS Accredited Organisation. For the current study, ECGs would be recorded routinely at the participating hospitals. The ECGs would be transferred to the local ECG management system. Wherever feasible, relevant files would be extracted in XML format and transferred in a secure, anonymised fashion to the ECG Core Lab in Glasgow Royal Infirmary for analysis using the University of Glasgow ECG Analysis Program which is used worldwide (122). Where this approach is not possible, copies of the ECGs will

be made and uploaded to the e-CRF portal. Records would be anonymised simply by inserting only a patient ID number together with age, sex and race of the individual.

ECG quality assurance: ECGs will be checked for completeness and quality and feedback will be provided accordingly by the core lab to local sites.

3.7.2.2 Coronary angiogram

Importance of coronary vascular function

Acute coronary thrombosis causes reductions in epicardial artery flow and microvascular perfusion. Coronary blood flow parameters can be measured objectively based on coronary angiography and the TIMI coronary flow grade (95)

Coronary angiogram acquisition

The coronary angiogram is the standard of care for primary PCI. Coronary angiograms will be performed in the usual way before reperfusion, to guide PCI and then again finally to document the final results of the procedure. Since the coronary guidewire will already be in place positioning of the aspiration catheter should be straightforward. No additional cine angiograms will be needed just fluoroscopy to guide and confirm the position of the aspiration catheter which should be advanced 1 – 2 cm proximal to the lesion for study drug infusion.

Angiographic views: A comprehensive angiogram should be obtained, in line with usual care, at the start and end of the PCI procedure. Orthogonal views of the culprit lesion should be obtained. For the purposes of the secondary angiographic outcomes, the exact same positions of the x-ray image intensifier should be used at the beginning and end of the PCI in order that matched comparisons of the angiographic images can be made by the core laboratory.

Coronary angiogram analysis

TIMI coronary flow grade

TIMI Coronary Flow Grade	Description
0	No flow
1	Minimal flow past obstruction
2	Slow (but complete) filling and slow clearance
3	Normal flow and clearance

The TIMI flow grade is straightforward to evaluate in the catheter laboratory and is independently predictive of prognosis (95).

Coronary angiography also provides other information on coronary blood flow and myocardial perfusion. For example, the TIMI blush grade provides an ordinal score for contrast washout at the end of each angiogram (96). The TIMI blush grade is also predictive of prognosis (97).

Tissue myocardial perfusion (blush) grade

Grade	Description
0	No myocardial blush
1	Minimal blush and very slow clearing (e.g. present at beginning of next cine)
2	Good blush with slow clearing of myocardial contrast (present at end of cine but gone at beginning of next)
3	Good blush and normal clearing (i.e. gone by end of cine)

TIMI frame count

The TIMI frame count is a simple objective continuous variable index of coronary blood flow, representing the amount of time (in frames) for contrast dye to reach a standardised distal landmark, corrected for vessel length (97). The corrected TIMI frame count is also predictive of prognosis (97).

Method: *Corrected TIMI frame count (CTFC, normal < 27 frames)*. The CTFC is the number of cine frames required for contrast to first reach standardized distal coronary landmarks in the culprit artery and is measured with a frame counter on a cine viewer. A frame count of 100, a value that is the 99th percentile of patent vessels, is imputed to an occluded vessel. CTFC is a measure of time, and data will convert when necessary according to film speed (e.g. 30 frames/s). The CTFC will be divided by 30 to calculate the transit time for dye to traverse the length of the artery to the landmark in seconds and multiplied by 1000 to calculate the time in milliseconds. This will be used along with the heart rate to calculate the fraction of a cardiac cycle required for dye to traverse the artery: fraction of cardiac cycle (CTFC/30 seconds)/(60s/heart rate). Calculation of the fraction of a cardiac cycle required for dye to traverse the culprit artery normalizes the CTFC for heart rate.

Thrombus grade

Thrombus burden revealed during coronary angiography can be classified according to the TIMI thrombus grade (98), which is straightforward to assess.

Grade	Description
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- 0 No cine-angiographic characteristics of thrombus are present
- 1 Possible thrombus is present, with such angiography characteristics as reduced contrast density, haziness, irregular lesion contour, or a smooth convex "meniscus" at the site of total occlusion suggestive but not diagnostic of thrombus
- 2 Definite thrombus, with greatest dimensions $\leq 1/2$ the vessel diameter
- 3 Definite thrombus but with greatest long axis dimension $> 1/2$ but < 2 vessel diameters
- 4 Definite thrombus, with the largest dimension ≥ 2 vessel diameters
- 5 Total occlusion

Thrombus area

Coronary thrombus area = thrombus length (mm) x reference diameter (mm) x diameter stenosis

Intra-procedural thrombotic events

An IPTE, defined as the development of new or increasing thrombus, abrupt vessel closure, no reflow, slow reflow, or distal embolisation at any time during the procedure (99).

Angiographic measurements

Angiography Core Lab (PIs Berry & McEntegart): Each coronary angiogram will be de-identified with the patient study number, and sent to the Glasgow Core Laboratory by electronic transfer, or where this is not possible, by CD/DVD. The angiogram will be analysed by trained radiographers in the Angiography Core Laboratory of the Golden Jubilee National Hospital under the direct supervision of Professors Berry and McEntegart who have considerable experience of angiographic core lab analyses (99, 125, 133). Dedicated angiographic software and image review workstations will be used.

Quality assurance of angiograms: Investigator training will be essential to ensure optimal angiographic data, including paired angiograms in matched positions after reperfusion and again at the end of PCI. A standard operating procedure for coronary angiography will be provided to local sites, included at site initiation. Feedback to local sites will be provided by the core lab throughout the recruitment period.

3.7.2.3 Biochemical assessment of infarct size with Troponin T and remodelling with NT-pro BNP

Blood samples (~20 ml) will be collected to measure high-sensitivity troponin T and NT-pro BNP. Serial measurements of troponin T using the Roche high-sensitivity assay will be used to provide a biochemical measurement of infarct size (area-under-the-curve) (1,134). Troponin T will be measured in blood samples collected at baseline (before study drug administration), and 2hr \pm 1, 24 hr \pm 12, 2-7 days, and 12 weeks (\pm 2 weeks) post-MI. NB Research blood tests at T 0, 2 hrs and 24 hrs are preferred but not compulsory. NT-pro BNP will be measured in blood samples collected at baseline, 2-7 days and 12 weeks \pm 2 in order to provide a biochemical measurement of left ventricular remodelling (within-subject change in NT-proBNP at follow-up from baseline (135)). The biochemical analyses will be performed in a central laboratory in the Glasgow. The biochemical measurements are justified since their measurement accuracy and coefficient of variation are lower than MRI therefore the biochemical parameters will provide independent measurements of infarct size and remodelling to complement the MRI data.

Local hospital blood sample handling

Blood samples will be handled according to a sample handling manual which will be provided to all sites. The blood samples for central laboratory analysis will be sent to: Dr Campbell Tait, Department of Haematology, MacEwan Building, 16 Alexandra Parade, Glasgow Royal Infirmary, G31 2ER.

Biochemistry Core Lab (PI Sattar) Serum and plasma samples will be stored at -80°C in the Glasgow Biorepository until batch analysis at the end of the study. High sensitivity Troponin T (134) and NT-proBNP (135) will be measured using standard assays e.g. the Elecsys 2010 electrochemiluminescence methods (Roche Diagnostics, Burgess Hill, United Kingdom) calibrated using the manufacturers reagents and an automated Cobas e411 analyser. For this assay, the lower limit of detection of Troponin T is 3 ng/L and the 99th percentile value in a healthy subpopulation is 14 ng/L (Roche Diagnostics, data on file). The between-assay coefficient of variations are 2.6% and 6.9% for control materials with mean Troponin T concentrations of 2378 ng/L and 29 ng/L, respectively.

For NT-proBNP, the low control coefficient of variation was 6.7% and the high control coefficient of variation was 4.9%. The troponin T and NT-proBNP results will be provided to the Robertson Centre for Biostatistics, University of Glasgow.

3.7.2.4 Coagulation (safety)

Haemostasis Central Lab (PI Tait) Blood samples (baseline – obtained after reperfusion but before administration of study drug therapy in the catheter laboratory; 2 hrs \pm 1, 24 hrs \pm 12 post-PCI in CCU) will be collected where feasible and plasma will be separated and stored for coagulation (136,137), according to the blood sample handling guideline. Research blood samples at these timepoints are preferred but not compulsory.

The coagulation laboratory analyses will be performed in the Haemostasis laboratory in Glasgow Royal Infirmary (Dr Campbell Tait, Department of Haematology, MacEwan Building 16 Alexandra Parade, Glasgow Royal Infirmary, Glasgow, G31 2ER) (102,103).

Activated clotting time (ACT) and haemoglobin measured in standard of care blood samples in local site laboratories will also be recorded in the case report form (see Section 3.7.3).

3.7.2.5 Thrombus histopathology (Sub-study)

Thrombus typically has a heterogeneous composition with fibrin strands binding a mixed cell population of red cells and platelets in varying amounts. The efficacy of thrombolysis will be influenced by the amount of fibrin. Fibrin rich thrombus is typically red whereas white thrombus is platelet rich. These will therefore be collected and assessed for % at the core laboratory. Samples will be collected/processed as per the Sample Handling Manual.

Histopathology central laboratory (PI: Wright, key collaborator) Therefore, the thrombus material that is aspirated in each case will be placed in formalin and sent to Dr Sylvia Wright, Consultant Pathologist, Department of Pathology, Queen Elizabeth University Hospital, Govan Road, Glasgow G51 4TF for histopathology and standardised assessment of fibrin content (100). The pathology samples will be handled and analysed according to laboratory guidelines.

3.7.2.6 Coronary physiology (Sub-study)

Sub-study of IMR and CFR(105-119) –guidewire-based coronary function testing will be during the PCI procedure in 256 participants (n=85 per group). The physiology study will begin after a minimum of 30 subjects have been randomised, and near-consecutive participants will be included. IMR and CFR will be measured according to an investigator guideline. Absolute coronary blood flow may be measured, at operator discretion.

3.7.2.7 Optical coherence tomography (Sub-study)

Sub-study of thrombus burden at the end of PCI (101-104)- OCT imaging will be performed at the end of the PCI in 90 participants (n=90 in total). OCT will be performed according to an investigator guideline.

3.7.2.8 Quality of life

Quality of life: will be assessed using the EQ5D-5l QoL questionnaire (138). The study participant will complete the questionnaire with a member of the research team (as needed) 2 -7 days and 12 weeks \pm 2 after randomisation (and at 52 weeks \pm 4, 104 weeks \pm 4 and at the final assessment). The EQ5D data will be uploaded and entered locally into the eCRF.

3.7.2.9 Assessment of health outcomes

Assessment of adverse events during initial hospitalisation

During the initial hospital stay patients will be assessed as per study schedule by the research team for serious adverse events (SAEs) and adverse events of interest (i.e. bleeding complications).

Assessment of adverse events during follow-up

Health outcomes will include death, re-hospitalisation for cardiovascular events including recurrent MI, heart failure, stroke/TIA, and acute bleeds (140-144). Data on health outcomes will be collected during the index admission (any event during the index admission will be recorded) and after discharge from each hospital by a clinic review for all patients at 3 months (including for the follow-up MRI scan) and telephone contact at 12 and 24 months and at the close of the study (or by letter or patient review as appropriate to ensure complete data, including for quality of life). Event reporting by the sites will be performed prospectively during the trial and trial unit quality assurance will also take place prospectively. In addition, in order to further support recording health outcomes, electronic case record linkage will be performed using the Community Health Index (CHI) number in Scotland and NHS number in England and Wales. The record linkage plan will be implemented by the Information and Statistics Division (ISD), and Clinical Practice Research Datalink (CPRD) linked to the Information Centre in England at the end of the study (33 months), and again at least 3 years for longer term follow-up, dependent on future funding. These are quality assured NHS systems made possible by electronic registration of all deaths and hospitalisations (and their causes) which have been used widely, including for studies including publications by the trial statistician (139). Patient follow-up will begin from the date the first patient is recruited till 12

months after the final patient is recruited (24 months in total; mean Follow Up anticipated to be 18 months).

3.7.2.10 Assessment of health economics

The economic evaluation will compare usual care (as defined by the control group arm of the RCT) with usual care-plus-alteplase. The perspective of the economic evaluation will be that of the NHS plus social services, reflecting the approach by NICE in technology appraisal.

Resource use data will be collected for:

- Days in hospital (including in each of the following ward types: intensive care, coronary care, and general cardiology ward)
- Medicines given to the patient (mainly alteplase but medicines such as glycoprotein IIb/IIIa)
- Costs of procedures (including the duration of PCI, need for repeat procedures, use of implantable defibrillators)
- Repeat hospital admissions related to the original STEMI event

Complications will be captured through added length of stay or through subsequent procedures carried out. We recognise the list above does not include follow-up care but this will be determined by the RCT protocol so we will test several different schedules for follow-up clinic visits in a sensitivity analysis.

We will obtain costs for this resource use from NHS Reference Costs, Scottish Health Service Costs and the PSSRU Publication "Unit Costs of Health and Social Care". We will calculate a cost per patient and report cost distributions.

We propose two forms of economic evaluation. First, we will use the difference in the trial primary outcome to estimate the added cost per unit change in MVO as a **cost-effectiveness analysis** using a time horizon of 18 months (the expected mean follow-up time). While this fits with the trial results interpretation may not be straightforward so we will also conduct an exploratory **cost-utility analysis** to estimate a *cost per QALY over the lifetime of a patient*. We can only explore the model because the trial is not powered to detect differences in either utility values (quality of life) or of survival so we will be using data where we may not have observed differences.

We anticipate an important benefit of MVO prevention will be in terms of avoiding cases of heart failure. Therefore we will explore whether the model should include states for absence of heart failure and varying severities of heart failure (e.g. NYHA class), as described by Goehler et al (145). If alteplase is not efficacious in terms of its primary and secondary outcomes then we will not complete the analysis because alteplase would be more expensive for no gain. However, assuming alteplase does achieve a gain that is statistically and clinically significant then we will proceed with the analysis.

3.7.3 Other assessments

3.7.3.1 Haematology and clinical chemistry

A complete blood count and U&E test for renal biochemistry will be performed at the start of primary PCI as part of standard care in the hospital laboratory. Since unfractionated heparin (5000 IU as per standard dosing) will be administered at the time of the first medical contact (e.g. Ambulance service staff, Accident and Emergency Department or cardiac catheterisation laboratory), an activated clotting time (ACT) will be performed in the catheter laboratory at the start of primary PCI to confirm initial therapeutic anticoagulation (target ACT 250 s) with heparin and the ACT will be rechecked at 20 min intervals to ensure therapeutic anticoagulation is maintained during the procedure

4. Study Treatment

4.1 *Alteplase*

4.1.1 Alteplase treatment schedule after reperfusion pre-stenting

Patients will be allocated to a treatment either control or active treatment as below:

Control Arm:	placebo vial and placebo vial
Arm A (very low dose):	alteplase 10mg vial and placebo vial
Arm B (low dose):	alteplase 10mg and alteplase 10mg vial

4.1.2 Rationale for chosen regimen

The rationale for an extended treatment period involving slow manual intracoronary infusion of study drug over 10 minutes is to maximise and sustain alteplase concentrations in the culprit artery and its microcirculation in order to effectively lysis of fibrin-rich thrombus.

4.1.3 Investigational Medicinal Product (IMP) administration

Alteplase 10mg and matched placebo will be supplied as lyophilisate powder. Each kit will contain two vials of study drug and two vials of solvent (10ml sterile water for injection) for reconstitution. The study drug will be administered at the start of the PCI procedure, after reperfusion, pre-stenting.

The IMP should be reconstituted immediately prior to use at the time of the PCI. The full volume of solvent (sterile water for injection) should be added to each alteplase/placebo vial. Any vigorous agitation should be avoided to prevent foam formation. Once frothing has subsided, 10ml from each reconstituted alteplase/placebo vial should be withdrawn into a single syringe for administration purposes. The solution for administration will have a maximum alteplase concentration of 1mg/ml.

The reconstituted solution is a clear and colourless to pale yellow solution. Prior to administration it should be inspected visually for particles. The reconstituted solution is for single use only. Any unused solution should be discarded. Further information on preparation and administration is provided in the study specific 'IMP Management and Accountability Manual'.

The alteplase/placebo solution (20ml) should be administered by slow manual intra-coronary infusion of up to 10 minutes but a minimum of 5 minutes. The slow infusion may be interrupted and restarted if clinically indicated at the discretion of the treating clinician however, any interruptions to administration must be kept to a minimum and should not exceed 30 minutes. Administration must be completed prior to stenting.

4.2 Study Drug Supplies

4.2.1 Supply & storage of study treatment

For the purpose of this study, alteplase is considered Investigational Medicinal Product (IMP). Alteplase and a matched placebo will be manufactured in accordance with Good Manufacturing Practice. The kit number will be allocated at the time of randomisation via IVRS/IWRS. The IMP and placebo will be supplied as powder and solvent for solution for infusion and will be reconstituted at the time of the PCI procedure. The undiluted vials will be stored as per the IMP labelling. The IMP kits will be distributed to participating hospital pharmacies for 'out of pharmacy' storage in the catheter laboratory for ease of access during primary PCI. Further information will be provided in the study specific 'IMP Management and Accountability Manual'.

4.2.2 Labelling of study treatment

The outer kit, alteplase 10mg or placebo and diluent vials will be labelled in accordance with local regulatory requirements.

4.2.3 Drug accountability

Only those supplies intended for use in the study should be supplied to study participants. A record of all study drug movements must be kept for accountability purposes. Drug accountability records for all used supplies will include IMP receipt and disposal. They must include date, kit number, batch number and expiry date of IMPs. These records should be maintained to adequately document that patients were provided with the doses specified in the protocol. These inventories must be made available for inspection by the study Sponsor or their designee and regulatory agency inspectors. Further information will be provided in the study specific IMP Management and Accountability Manual.

4.2.4 Management of bleeding after alteplase/placebo administration

Should serious bleeding occur then the risk of bleeding, in particular cerebral haemorrhage needs to be considered against the benefit of anti-coagulation which is standard treatment in primary PCI for coronary thrombosis. Alteplase is metabolized in the liver and the circulating (systemic) half-life of alteplase correlates with the physiological effects of the drug. The circulating half-life is approximately 5 minutes therefore bleeding events that occur more than 5 half-lives (i.e. hours later) may not be associated with clinically meaningful circulating concentrations of alteplase. This is all the more relevant in T-TIME since the study drug is administered locally into the culprit artery and its branches with fibrin-containing thrombus. The study drug is not administered directly into the systemic circulation, and because of slow local intra-coronary administration over 5-10 minutes, the release of alteplase via venous effluent flow into the systemic circulation is minimized. Therefore, unblinding for a study participant who experiences a bleed hours after study drug administration may have qualified value. The attending doctor will make this decision, if necessary after discussion with the CI.

If the cardiologist feels that the risk from bleeding outweighs the risk from coronary thrombosis then concomitant administration of heparin should be stopped and potentially reversed by administration of protamine if heparin has been administered within 4 hours before the onset of bleeding. In the few patients who fail to respond to these conservative measures, judicious use of transfusion products may be indicated. Transfusion of cryoprecipitate, fresh frozen plasma, and platelets should be considered with clinical and laboratory reassessment after each administration. A target fibrinogen level of 1 g/l is desirable with cryoprecipitate infusion. Anti-fibrinolytic agents may be used as a last alternative after discussion with the duty Consultant Haematologist.

If major bleeding does occur following discussion with the duty Consultant Haematologist, management could include:

1. Local pressure if possible
2. Fresh frozen plasma 12 ml/Kg (may be followed by 5-10 units (1-2 bags) of cryoprecipitate once coagulation parameters and the fibrinogen level are known from local haematology laboratory. Fresh frozen plasma and cryoprecipitate will provide a source of fibrinogen as well as other potentially depleted factors (FV and FVIII)
3. Administer 1 - 2 platelet transfusions to overcome the anti-platelet of these treatments
4. Infuse 10-15 mg/kg IV of tranexamic acid over 20 minutes initially (risk of thrombosis or re-MI is uncertain, but may be quite low) then 1 mg/kg/hour continuous infusion for 6 - 10 hours. Involvement of a Haematologist is recommended. N.B. Tranexamic acid is contra-indicated in patients with epilepsy.
5. Consider other interventions as appropriate to the site of bleeding.

N.B. If the bleed occurs >24 hours after study drug administration then any residual thrombolytic effect at this stage should be minimal so reversal of anti-platelet effect and anti-coagulant effect should be considered rather than cryoprecipitate and tranexamic acid.

Type & duration of follow-up of subjects after adverse events. The study team will remain in close contact with patients and their clinical teams whenever an adverse event has occurred.

PROTOCOL FOR MANAGEMENT OF ADVERSE EVENTS: Any bleeding complications will be managed as clinically appropriate including with direct pressure for compressible bleeding points or administration of coagulation factors (but only after discussion with an expert haematologist on-call in each hospital). Allergic reactions following alteplase are very rare.

The procedure for the assessment and reporting of adverse events is detailed in Section 5 below.

4.3 Unblinding procedure

Breaking of the study blind should only be performed where knowledge of the treatment is absolutely necessary for further management of the patient. Emergency unblinding will be via an IVRS telephone menu system and will be available at all times. Several prompts in the system warn the user that they require to be a health professional and to record their name. For each unblinding request an email alert is generated to the Project Manager and Chief Investigator (CI). A clinician information sheet will be inserted into the patient's medical notes which will include information on emergency unblinding and local PI contacts should the patient be transferred to another hospital after the point of verbal consent.

Patients will be provided with an alert card at a convenient time after verbal consent is obtained, when they are provided with the patient information sheet. They will be asked to carry the alert card with them at all times and to show the card to any doctors or healthcare professionals who are involved in their care. Patients will also be asked at the 2 -7 day scanning visit if they still have this and be provided with another if required. The Patient Alert Card will be collected from patients at the end of their involvement in the study.

The sponsor retains the right to unblind the treatment allocation in order to report suspected adverse events to the regulatory authorities.

4.4 Optimal standardised anti-thrombotic therapy

4.4.1 Anti-thrombotic therapy at 'first medical contact'

Patients whose pre- or initial in-hospital care is coordinated by the Ambulance Service, General Practitioner and/or Emergency Care Physicians should receive optimal anti-thrombotic treatment according to local protocols (1). It must include an anticoagulant product and an anti-platelet product. An example of an anti-thrombotic regimen prior to arrival in the catheterization laboratory is as follows:

300 mg of aspirin orally, 600 mg of clopidogrel orally (180 mg of ticagrelor or 60 mg of prasugrel if age <75 years and > 60 kg), as per local practice 5000 IU of unfractionated heparin I.V. (time of administration to be recorded)

4.4.2 Anti-thrombotic therapy during primary PCI

After the first medical contact, on arrival in the catheter laboratory the activated clotting time (ACT) will be measured at the start of primary PCI to confirm initial therapeutic anticoagulation (target ACT 250 s) with unfractionated heparin. In order to standardise anticoagulation and avoid excess risk of bleeding, if the ACT is < 250, a further dose of heparin should be administered to establish immediate anticoagulation.

In line with optimal NHS care, anti-coagulation in the catheter laboratory will be maintained with heparin.

Glycoprotein IIb/IIIa therapy

GpIIb/IIIa inhibitor therapy should only be administered for 'bail-out' as per clinical guidelines (1). The indications for bail-out GpIIb/IIIa inhibitor therapy include angiographic evidence of massive thrombus, slow or no-reflow, or a thrombotic complication) (1). The choice of gpIIb/IIIa inhibitor is as per local practice. The recommended dose for abciximab is 0.25 mg/kg intravenous bolus, followed by a continuous intravenous infusion of 0.125 microgram/kg per min (to a maximum of 10 microgram/min) for 12 hours. The recommended dose for tirofiban (Aggrastat) is 25 microgram/kg given as a bolus followed by an intravenous infusion of 0.15 microgram/kg/min for up to 24 hours.

If not already administered, loading doses of dual oral anti-platelet drugs (see 4.4.1) should be given at the start of the primary PCI procedure.

4.4.3 Anti-thrombotic maintenance therapy after primary PCI

The majority of patients are expected to be receive aspirin plus an anti-platelet as the post PCI dual anti-thrombotic maintenance therapy. Local NHS prescribing guidelines may cause

variations in the use of these drugs between participating sites. Examples of dual anti-platelet therapy are:

75 mg of aspirin daily continuously

Plus 90 mg twice daily of ticagrelor or 10 mg daily or prasugrel (5 mg daily if age > 75 years) or clopidogrel 150mg for one week then 75 mg daily with the duration of therapy according to local prescribing guidelines.

In line with optimal standards of care, for patients switching from clopidogrel to either ticagrelor or prasugrel, a loading dose should be initially administered (see 4.4.1). In hospitals in which clopidogrel remains the standard of care, this drug should be continued up to 1 year, as clinically appropriate and as part of standard care. Low molecular weight heparin (e.g. 40 mg of enoxaparin or 20 mg in patients with renal failure) should be given daily for thromboprophylaxis.

5. PHARMACOVIGILANCE

5.1 *Definitions of adverse events*

Adverse Event (AE) – Any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR) – Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Any adverse event or adverse reaction that:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- consists of a congenital anomaly or birth defect
- is otherwise considered medically significant by the investigator

i.e. Important adverse events/ reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Suspected Serious Adverse Reaction (SSAR)

Any adverse reaction that is classed in nature as serious and which is consistent with the information about the medicinal product in question set out in the summary of product characteristics (SmPC) or the Investigator's Brochure (IB).

Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any adverse reaction that is classed in nature as serious and which is **not** consistent with the information about the medicinal product in question set out in the SmPC or the Investigator's Brochure (IB).

5.2 *Assessment, recording and reporting of Adverse Events*

All adverse events (AEs) of interest will be recorded in the eCRF but only serious adverse events (SAEs as defined above) will be notified to the sponsor, assessed, reported, analysed and managed in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004 (as amended) and this protocol. The mechanism for this process will be based on reporting through the eCRF.

Full details of all AEs of interest will be recorded in the eCRF. These AEs will be monitored and followed up until satisfactory resolution or stabilisation.

All SAEs will be notified to the Sponsor. Furthermore, specific AEs of interest and relevant to the intervention are defined below:

We will collect information on clinically important adverse events (e.g. bleeds), those related to the drug, and all SAEs. The SAEs, all of which will be notified to the Sponsor, to be included are:

As the serious adverse events eCRF is used to identify clinical end points for the duration of the follow up a distinction will be made between those events relevant for Pharmacovigilance and those required for the collection of end point data. The detail of this is captured below in the section titled Recording and Reporting of SAEs but to summarise:

- All SAEs submitted within the period from the time of consent until 30 days post study drug will be subject to the Medicines for Human Use (Clinical Trials) Regulations 2004 and as such will be reported to the sponsor within 24 hours of the investigators becoming aware of the event. All SAEs will be assessed for causality and expectedness by the principal investigators at each trial site, with oversight by the chief investigator. These events will be used to assess the safety of the drug and subject to expedited submission to the MHRA and REC if the event is classed as related to the study drug and unexpected. The events occurring during this period of time will also be assessed by the CI as potential clinical endpoints. These events will be used to inform the developmental safety update report.
- SAEs reported outside of the period from consent until 30 days post randomisation will not be used for the purposes of Pharmacovigilance; rather for the collection of clinical endpoints. As such these events do not require a formal assessment for causality and expectedness or require expedited reporting. Trial sites will be asked to report any SAEs between the follow up times defined within this protocol; that is; from 30 days post treatment until the 3 month follow up, 12 month follow up, 24 month follow up or trial close out (the earlier of these two dates)

Please note: If the PI believes that a serious adverse event following 30 days post treatment is suspected to be related to the trial drug and is unexpected this event must be reported

within 24 hours and must be assessed for causality and expectedness by the principal investigators at each trial site, with oversight by the chief investigator.

SAEs RELEVANT FOR EFFICACY AND SAFETY ANALYSES

Clinical event committee (CEC)

A CEC will be established to provide independent adjudication of serious adverse events using de-identified clinical information to be provided by the Chief Investigator/Clinical Trials Unit. The committee will be independent and include at least 3 cardiologists who are independent of the study. A CEC charter will be established with standardised definitions for clinical endpoints according to the "*Standardised definitions for endpoint events in cardiovascular trials*" (FDA, 138) and the "*Third Universal Definition of Myocardial Infarction*" (134). CEC assessments and reporting will take place prospectively as the trial progresses.

Definitions

1) **Major Adverse Cardiovascular Events (Cardiovascular MACCE)** is the composite of 'cardiovascular death, non-fatal MI, unplanned hospitalisation for TIA or stroke'

PCI and CABG are adverse events but are not defined as 'major'.

2) **Major Adverse Cardiac Events (Cardiac MACE)** are defined as 'cardiac death, non-fatal MI or unplanned hospitalisation for heart failure'

The Cardiac MACE will be considered for all MIs and also for MACE with spontaneous MI only (i.e. not Type 4 or Type 5 MI).

3) MI associated with revascularisation procedures (types 4 and 5) (141)

Type 4a: Myocardial infarction related to percutaneous coronary intervention (PCI)

Myocardial infarction associated with PCI is arbitrarily defined by elevation of cardiac troponin values $>5 \times 99$ th percentile URL in patients with normal baseline values ≤ 99 th percentile URL) or a rise of cardiac troponin values $>20\%$ if the baseline values are elevated and are stable or falling. In addition, either (i) symptoms suggestive of myocardial ischaemia, or (ii) new ischaemic ECG changes or new LBBB, or (iii) angiographic loss of patency of a major coronary artery or a side branch or persistent slow or no-flow or embolisation, or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.

Type 4b: Myocardial infarction related to stent thrombosis

Myocardial infarction associated with stent thrombosis is detected by coronary angiography or autopsy in the setting of myocardial ischaemia and with a rise and/or fall of cardiac biomarkers values with at least one value above the 99th percentile URL.

Type 5: Myocardial infarction related to coronary artery bypass graft

Myocardial infarction associated with CABG is arbitrarily defined by elevation of cardiac biomarker values $>10 \times$ 99th percentile URL in patients with normal baseline cardiac troponin values \leq 99th percentile URL. In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Assessment of efficacy

Other SAEs for the CEC charter: RECURRENT MI will be defined according to the Universal Definition of MI (141) and will involve ischaemic symptoms (e.g. chest pain) > 20 min with new ECG changes (new Q waves and/or ST segment elevation $>0.1\text{mV}$ in 2 leads for > 30 min), a cardiac biomarker elevation (>99 th centile upper reference limit). For patients who die, new chest pain or ST elevation would fulfil the criteria for MI. CEREBROVASCULAR EVENTS: TRANSIENT ISCHAEMIC ATTACK (TIA) is any focal neurological deficit with sudden onset that resolves within 24 h. A neurology review should be obtained wherever possible. STROKE is any rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting >24 hours or leading to death with no apparent cause other than a vascular origin (142). HEART FAILURE: Within the index admission will be scored according to Killip's classification (143); Heart failure after discharge will be defined as a hospital admission with NYHA class III or IV heart failure and intravenous diuretic therapy or an increase in oral diuretics. SAEs obtained through Information and Statistics Division (ISD) of NHS Scotland and the Clinical Practice Research Datalink (CPRD) with the NHS number will be based on ICD-10 codes. Serious adverse events and those of interest (i.e. bleeding) will be collected by the research team (clinical research fellow, research nurse). Details of the event will be added to the eCRF. The sponsor PV Office will be automatically advised by the eCRF-based system of any SAEs, and those obtained from ISD through electronic case record linkage.

Assessment of safety:

The CEC will also consider serious adverse events for safety. Clinical assessments will be assessed prospectively during the index admission and after discharge will be performed

continuously by the Study Team (clinicians, research fellow, research nurse) as per the Schedule of Assessments. Information on adverse events will be supported by observations recorded by NHS doctors and nurses in the case notes during routine care. The safety assessments will evaluate:

- (1) Acute bleeds, as defined by the Bleeding Academic Research Consortium (BARC) standardised definitions for clinical trials (50).
- (2) All-cause mortality and rehospitalisation for MI, or heart failure, revascularisation or stroke;
- (3) Fibrinogen concentration ≤ 24 h post-MI. As described in the TIMI 10A study (43), a significant fall in circulating FIBRINOGEN concentration (e.g. $>50\%$ reduction at 2 h and/or 24 h vs. baseline (43)) in systemic blood samples could infer systemic fibrinolysis (which we do not expect to see with reduced dose alteplase) which would increase bleeding risk and therefore represent a surrogate safety marker. For this reason, the IDMC will assess the results of the coagulation analyses after 10% of the patients have been recruited.

When a serious adverse event occurs (e.g. death, major/severe bleeding Type 3 – 5, as per BARC criteria), the event will be reported by the study team within 24 h to the coordinating centre using the eCRF. The TIMING of safety assessments will correspond with those for EFFICACY (as outlined in 8.1).

This is because of the relatively low rate of major bleeding and the modest sample size in the trial.

Bleeding

In line with recent developments to standardise the definition of bleeding the standardised bleeding definitions of the Bleeding Academic Research Consortium (BARC) will be adopted (50).

Table 1. Bleeding Academic Research Consortium Definitions (BARC) for Bleeding (50).

Type	Definition
0	No bleeding
1	Bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a healthcare professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a healthcare professional
2	Any overt, actionable sign of haemorrhage (eg, more bleeding than would be expected for a clinical circumstance, including bleeding

	<p>found by imaging alone) that does not fit the criteria for type 3, 4, or 5 but does meet at least one of the following criteria:</p> <p>(1) requiring nonsurgical, medical intervention by a healthcare professional,</p> <p>(2) leading to hospitalization or increased level of care, or</p> <p>(3) prompting evaluation</p>
3a	<p>Overt bleeding plus haemoglobin drop of 3 to 5 g/dL* (provided haemoglobin drop is related to bleed)</p> <p>Any transfusion with overt bleeding</p>
3b	<p>Overt bleeding plus haemoglobin drop 5 g/dL* (provided haemoglobin drop is related to bleed)</p> <p>Cardiac tamponade</p> <p>Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/haemorrhoid)</p> <p>Bleeding requiring intravenous vasoactive agents</p>
3c	<p>Intracranial haemorrhage (does not include microbleeds or haemorrhagic transformation, does include intra-spinal)</p> <p>Subcategories confirmed by autopsy or imaging or lumbar puncture</p> <p>Intraocular bleed compromising vision</p>
4	<p>CABG-related bleeding</p> <p>Perioperative intracranial bleeding within 48 h</p> <p>Reoperation after closure of sternotomy for the purpose of controlling bleeding</p> <p>Transfusion of 5 U whole blood or packed red blood cells within a 48-h period</p> <p>Chest tube output 2L within a 24-h period</p>
5	Fatal bleeding
5a	Probable fatal bleeding; no autopsy or imaging confirmation but clinically suspicious
5b	Definite fatal bleeding; overt bleeding or autopsy or imaging confirmation

CABG indicates coronary artery bypass graft. *Corrected for transfusion (1 U packed red blood cells or 1 U whole blood 1 g/dL haemoglobin).

†Cell saver products are not counted

Bleeds that fulfil the BARC Type 3 – 5 criteria are taken to represent major bleeds [50]. Non-major bleeding i.e. Type 0 or Type 1 BARC criteria will not be recorded as SAEs (and so would not be reportable to the sponsor) but would be recorded in the eCRF.

Clinical assessments will be assessed prospectively during the index admission and after discharge will be performed continuously by the Study Team (clinicians, research fellow, research nurse) as per the Schedule of Assessments. Information on adverse events will be supported by observations recorded by NHS doctors and nurses in the case notes during routine care.

In contemporary practice the rate of acute severe bleeding, such as defined by the ACUITY (52) and GUSTO bleeding criteria (106), is low (<1%) in acute coronary syndrome patients undergoing PCI, especially those in whom radial artery access is used as in the RIVAL trial (51).

In the interests of patient safety, radial artery access is increasingly the preferred route of arterial access for PCI in UK hospitals. In the Lead Site (Golden Jubilee National Hospital), radial artery access is used in primary PCI in >90% of cases. The IDMC may also wish to consider coagulation results (e.g. fibrinogen concentrations (43)) in any patient who has had a bleed in order to interpret whether or not the bleeding event might be related to predisposition to bleeding related to study drug therapy.

Assessment of adverse events

All adverse events must be assessed for seriousness. SAEs occurring between the date of consent and 30 days post treatment must be assessed for causality, expectedness and severity and notified to the Sponsor. This is the responsibility of the CI or designee.

SAEs occurring more than 30 days post treatment do not require assessment of causality and expectedness as they form potential clinical endpoints and are outside the pharmacovigilance reporting requirements.

SAEs that are potentially relevant to the designated secondary health outcomes will be assessed by a Clinical Event Committee.

Please note: If the PI believes any serious adverse event following 30 days post treatment is suspected to be related to the trial drug and is unexpected this event must be reported within 24 hours and must be assessed for causality and expectedness by the principal investigators at each trial site, with oversight by the chief investigator.

Assessment of seriousness

See definitions in 5.1 above

Assessment of causality i.e. does the event have a “reasonable causal relationship” with trial medication. The following categories are used:

None: The event is not considered to be related to the study drug.

Possible: Although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship makes other explanations possible.

Probable: The temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.

Definite: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that the study drug is the most likely cause.

Assessment of expectedness.

If an SAE is considered to be related (possibly, probably or definitely) to the study medication, an assessment should be made of the expectedness of the reaction i.e. is the reaction a recognised adverse effect of the medication.

The expectedness of an adverse reaction is assessed against the Reference Safety Information i.e. the list of expected reactions detailed in the Summary of Product Characteristics (SmPC) for the Investigational Medicinal Product approved during Clinical Trial Authorisation process.

Expected: consistent with the relevant product information documented in the RSI.

Unexpected: not consistent with the relevant product information documented in the RSI.

Assessment of Severity

This should be assessed and described using the following categories:

- Mild-awareness of event but easily tolerated
- Moderate-discomfort enough to cause some interference with usual activity
- Severe-inability to carry out usual activity.

Recording and Reporting of SAEs occurring between consent and 30 days post treatment

All SAEs arising from the date of consent until 30 days post treatment will be reported to the sponsor by entering the details into the eCRF as soon as reasonably practicable and in any event within 24 hours of first becoming aware of the event. Any follow-up information should also be reported.

If SAE reporting is not possible via the eCRF, a paper SAE form should be completed and forwarded to the sponsor to ensure compliance with the 24 hour reporting requirement.

The SAE form is available at <http://www.glasgowctu.org/complete-paper-sae.aspx>. The form is downloaded, printed, completed, signed and forwarded to the sponsor (Pharmacovigilance Office) by fax or email.

Pharmacovigilance Fax: +44(0)141 357 5588

Pharmacovigilance email: pharmacovig@glasgowctu.org.

If necessary a verbal report can be provided by contacting the Pharmacovigilance Office at +44(0)141 330 4744. A verbal report must be followed up as soon as possible with a signed written (or electronic) report.

SAE details will be transferred to the Glasgow Pharmacovigilance database.

SAEs that occur at any time after the inclusion of the subject in the study (defined as the time when the subject signs the informed consent) up to 30 days after the subject received the study drug will be reported.

SAEs reported occurring more than 30 days post treatment

SAEs occurring after the 30 day safety reporting period will be submitted at months 3, 12 and 24 and should be submitted via eCRF only as they are not subject to expedited reporting.

Reporting of SUSARs

All SUSARs will be reported in an expedited fashion to the MHRA and Ethics Committee

Fatal or life threatening SUSARs: not later than 7 days after the sponsor had information that the case fulfilled the criteria for a fatal or life threatening SUSAR, and any follow up information within a further 8 days.

All other SUSARs: not later than 15 days after the sponsor had information that the case fulfilled the criteria for a SUSAR

Unblinding Procedures for SUSAR reporting

In the event of a potential SUSAR, the treatment allocation will be unblinded by the sponsor before reporting to the MHRA and REC. SUSAR reporting to the participating investigators will be blinded. Study specific procedures will be developed.

The Pharmacovigilance office will report SUSARs to the MHRA on behalf of the CI via the MHRA eSUSAR reporting system and to Ethics committee by email.

Pregnancy

Any pregnancy occurring in a female subject or female partner of a male subject who becomes pregnant while participating in the Trial will be reported by the CI (or designee) to the PV office (sponsor) using the sponsor pregnancy reporting form (available at

www.glasgowctu.org) within two weeks of the CI first becoming aware of the pregnancy. The subject will also be followed to determine the outcome of the pregnancy and follow-up information forwarded to the PV office. Any resulting SAEs should be reported as per SAE reporting procedure above.

Study completion

The subject is considered to have completed the study EITHER after the completion of the last visit or contact (e.g. phone contact with the investigator or designee), OR after the last dose of the study medication, whichever is later. The date of discontinuation is when a subject and/or investigator determine that the subject can no longer comply with the requirements for any further study visits or evaluations.

5.3 Annual safety reporting

An annual safety report will be submitted to MHRA and REC as soon as is practicable and within 60 days of the anniversary of the issue of the Clinical Trials Authorisation. This report must be in the Development Safety Update Report (DSUR) format.

The CI will prepare and submit this report in liaison with the Pharmacovigilance Office. DSURs will be submitted until the End of the Trial as defined below.

6. STATISTICS AND DATA ANALYSIS PLAN

6.1 *Statistical analysis plan*

The T-TIME study will have a comprehensive Statistical Analysis Plan, which will govern all statistical aspects of the study, and will be authored by the Trial Statistician and agreed by the Trial Steering Committee before any unblinded data is seen.

6.2 *General considerations*

Null hypothesis: that the amount of MVO 2 days post-MI as revealed by cardiac MRI is similar in the alteplase groups compared to the placebo group.

Statistical approach: It is highly improbable that the low dose could be effective and the higher would not be. Therefore, to optimise the power of the study to show an effect of alteplase, the statistical design for the analysis of the primary outcome involves a comparison of the amount of LATE MVO on the DAY 2 -7 MRI SCAN between the higher dose group vs. placebo, and if this test is significant then the lower dose group will be compared vs. placebo. This strategy relates only to the formal assessment of whether or not the study is considered positive for the primary outcome. In reality, all between group comparisons will be performed i.e. high dose alteplase vs. placebo, low dose alteplase vs. placebo, all alteplase-treated patients vs. placebo, regardless of the treatment effect on the primary/secondary outcomes of the high dose group vs. placebo group. The statistical analysis will focus on estimation of the treatment effect and confidence intervals for all groups/comparisons rather than p-values. The trend test and non-parametric tests are intended as supporting analyses, and not for the pre-specified primary analysis.

Approximately half of the patients are expected not to have evidence of LATE MVO on late gadolinium enhancement MRI, however, the amount of LATE MVO is preferred for the primary outcome. From a statistical point of view, MVO expressed as a continuous trait will be highly skewed and with zero values recorded for patients without MVO (around half of the study participants), the MVO values will require some form of transformation and potentially a non-parametric method of analysis. For these reasons, late MVO as a binary variable (present/absent) is adopted as a prioritised secondary analysis in T-TIME.

All of these analyses will be reported in study publications to inform the design of any future larger multicentre study. In particular, a direct comparison of the two study doses will be an important secondary outcome, as will an additional exploratory analysis comparing the

combined alteplase arms with placebo. Given SAFETY is a prioritized outcome, we are motivated to establish the lowest effective dose, hence the dose-ranging design in our trial.

6.3 Primary efficacy variable

A Mann Whitney test or appropriate regression model will be used to compare % MVO between treatment groups, whilst a chi-square test or logistic regression model will be used to compare the proportions with any MVO, with logistic regression being used to calculate confidence intervals. Since there are 3 groups (alteplase 20mg, alteplase 10mg and placebo), we will first compare alteplase 20 mg vs. placebo with $p < 0.05$ required for significance. If this test is significant we will then test alteplase 10 mg vs. placebo. This hierarchical approach will conserve the overall type I error at 5%. Hence we will require a minimum of 558 patients with data for late MVO on the Day 2 -7 MRI scan. To allow for deaths and intolerance of MRI (e.g. claustrophobia) we will recruit 618 patients ($n=206/\text{group}$). The sample size will be sufficiently large to address feasibility, safety and efficacy. However, since efficacy, safety and ease-of-use will all be important when considering the approach to alteplase administration, information on these parameters will be evaluated to form an overall strategic view to select one for the future Phase III trial.

6.4 Secondary efficacy analysis

Between group differences in infarct size & myocardial salvage as quantitative traits (% of LV) will be assessed using appropriate general linear models adjusting for initial area-at-risk (% of LV). Other continuous outcomes will be analysed in a similar fashion where the assumptions of linear modelling are sufficiently met, and adjusted for baseline data. Where data are clearly not normally distributed (e.g. laboratory variables) or modelling assumptions are not met, standard transformations will be applied to address these issues. CLINICAL OUTCOMES will be presented with Kaplan-Meier time-to-event curves and compared where appropriate using hazard ratios and confidence intervals from Cox proportional hazards regression. The angiographic parameters will be assessed for association with clinical outcomes (96-99).

6.5 Safety analysis

Adverse events will be tabulated split by treatment group according to MedDRA system organ class (SOC) and preferred term (PT). Incidence of bleeds and other categorical outcomes will be summarised as counts and percentages and compared using Fisher's Exact tests. The primary criterion for safety monitoring would be the incidence of major bleeding events

(details to be determined in discussion with the IDMC before starting the study). The safety aspect will focus on major bleeds including a bleeding score (i.e. BARC (50)) to be agreed by the IDMC.

INTERIM SAFETY ANALYSIS: In order to assess for unwanted effects of alteplase on systemic fibrinolysis, the coagulation results and bleeding events, if any, of the first 10% of the study participants will be reviewed by the IDMC.

We plan to report SAEs to the IDMC (especially major bleeds (BARC Types 3 - 5, Table 1)) as they occur. We will do this by generating an electronic summary derived from the eCRF and sending the report electronically to minimise delay.

6.6 *Software and statistical analysis*

Clinical data will be made available to the data coordinating centre (the Robertson Centre for Biostatistics) through the web-based eCRF. The RCB is an NIHR-approved Clinical Trials Unit (Registration number 16). The statistical software to be used will be SAS and/or R and/or SPlus.

6.7 *Sample size*

SAMPLE SIZE: We plan a multi-centre study and 618 patients will be recruited. 206 subjects will be included in each of the 3 groups with study drug administration at the start of PCI after reperfusion:

1. Placebo
2. Alteplase 10 mg
3. Alteplase 20 mg

POWER CALCULATION AND ALTERNATIVE HYPOTHESIS:

A sample size of 618 (minimum 186/group) would result in 80% and 90% power to detect between-group mean differences of 1.49% or 1.72% respectively assuming mean (SD) of 3.2 (5.1)% for the extent of late MVO in the comparator group.

If the proportion of patients in the control (placebo) group with MVO is 47% then a 15% absolute reduction in the rate of MVO (i.e. 32% of patients with MVO) in the alteplase group could be detected with 80% power at a 5% level of significance with a minimum of 186 patients in each group. This estimated reduction in MVO is taken to be clinically meaningful. Allowing for incomplete data (e.g. incomplete MRI scans due to claustrophobia) and loss to follow-up, 618 patients will be randomised.

If the incidences of LATE MVO 2-7 days post-MI were 47% on placebo, 36% on low dose and 32% on high dose then there would be 80% power, and the power is 90% when both low dose and high dose have 32% event rates.

Pilot Data: We have based our sample size on local MRI data for MVO revealed by MRI 2 -7 days post-STEMI in patients selected according to the inclusion and exclusion criteria in T-TIME. Of ~1000 all comers STEMI treated by primary PCI in the Golden Jubilee National Hospital (May 2011 – November 2012), 302 were enrolled into an MRI cohort study (MR-MI study, <http://clinicaltrials.gov/ct2/show/NCT02072850>). 141 (47%) of these patients fulfilled the inclusion criteria for T-TIME of whom 96 (32%) had none of the exclusion criteria. Of these 96 patients, 45 (n=47%) had late MVO disclosed by contrast-enhanced MRI 2-7 days post-MI. The estimated incidence of late MVO affecting 47% of the control group is consistent with similar observations (46%-78%) in previous studies (3,17,18,64-72), and an estimated potential treatment effect on late MVO (16-18,72).

Similarly, the amount of MVO was measured in the MR-MI study. Of the patients who fulfilled the eligibility criteria for T-TIME the amount of LATE MVO (%LV mass) was: mean (SD) = 3.2 (5.1); median (IQR) = 0.9 (0, 3.7).

SECONDARY OUTCOMES: (1) INFARCT SIZE at 3 months (single time-point): 165 patients/group (n=550 incl. loss-to-FUp) would be sufficient to detect a between group difference in infarct size of 5.0% and a common SD of 11.6% (97% power; alpha 0.05). (2) For an absolute difference of 2.3% in DELTA EJECTION FRACTION (EF) at follow-up compared to baseline between the high-dose alteplase group & the placebo group, a common SD of 6.2% then 154 subjects/group (n=462; n=507 incl. 10% incomplete data) would be needed (90% power, alpha 0.05).

CORONARY PHYSIOLOGY SUB-STUDY: Based on pilot data from the BHF MR-MI study (110) if 256 patients have IMR measured at the end of PCI (allowing n≈10% for incomplete data, 232 subjects with IMR data) then for a comparison of IMR between two groups (placebo vs. alteplase (10 mg and 20 mg groups combined)), and given that the mean and standard deviation of IMR are 33.9 and 25.2 respectively, then there would be 85% power to detect a difference of 10 units (IMR) with an alpha of 0.05. For a comparison of IMR between 3 groups (placebo vs. alteplase 10 mg vs. alteplase 20 mg), and the mean differences in IMR between the 10 mg and 20 mg alteplase groups vs. placebo are 10 and 20 respectively, and the mean and SD of IMR in the placebo group are unchanged, then 108 subjects with complete data (n=36/group) would be needed, with 85% power.

Considering CFR, if 170 patients have CFR measured at the end of PCI (allowing n≈10% for incomplete data, 150 subjects with CFR data) then for a comparison of CFR between two groups (placebo vs. alteplase (10 mg and 20 mg groups combined)), and given that the mean

and standard deviation of CFR are 1.649 and 0.797 respectively, then there would be 85% power to detect a difference of 0.4 units (half a SD) with an alpha of 0.05. For a comparison of CFR between 3 groups (placebo vs. alteplase 10 mg vs. alteplase 20 mg), and the mean differences in CFR between the 10 mg and 20 mg alteplase groups vs. placebo are 0.4 and 0.8 respectively, and the mean and SD of CFR in the placebo group are unchanged, then 69 subjects with complete data (n=23/group) would be needed, with 85% power.

OCT SUB-STUDY: Based on 26 patients (allowing n=4, >10% for incomplete data) for comparison of two groups, there would be 80% power to detect a difference of 0.792 of a standard deviation (2.456 for an SD=3.1 and 0.238 for an SD of 0.3). For 90% power the minimum detectable between-group difference would be 0.917 of a standard deviation.

We have discussed these estimates with other cardiologist investigators in T-TIME and, based on our understanding of the literature and our experience in clinical practice, we believe that these projected EFFECT SIZES would potentially be clinically meaningful and credible.

6.8 Pre-specified analyses

We will undertake focused analyses in relation to patient characteristics that might be relevant to the efficacy and safety of the study intervention. The characteristics of interest include age, history of current cigarette smoking, presenting characteristics e.g. ischaemic time, coronary artery patency at initial angiography, and anti-thrombotic therapy.

Smoking status will be defined as per the internationally established definitions (https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm (146))

Current smoker: An adult who has smoked 100 cigarettes in his or her lifetime and who currently smokes cigarettes. Beginning in 1991 this group was divided into “everyday” smokers or “some days” smokers.

Never smoker: An adult who has never smoked, or who has smoked less than 100 cigarettes in his or her lifetime.

Former smoker: An adult who has smoked at least 100 cigarettes in his or her lifetime but who had quit smoking at the time of interview.

Some days smoker: An adult who has smoked at least 100 cigarettes in his or her lifetime, who smokes now, but does not smoke every day. Previously called an “occasional smoker”.

Every day smoker: An adult who has smoked at least 100 cigarettes in his or her lifetime, and who now smokes every day. Previously called a “regular smoker”.

6.9 Health Economics

The health economic analysis will reflect the approach by NICE in technology appraisal and will include resource use data (days in hospital, medicines, cost of procedures, repeat hospital admissions), adverse events, length of hospital stay and quality of life.

7. TRIAL CLOSURE / DEFINITION OF END OF TRIAL

The trial will end when the steering committee agrees that one or more of the following situations applies:

The planned sample size has been achieved;

The Independent Data Monitoring Committee (IDMC) has advised discontinuation, e.g. because of safety concerns about the trial, or a statistically significant difference in clinical outcomes is evident between the two treatment arms;

There is insufficient funding to support further recruitment, and no reasonable prospect of additional support being obtained;

New information makes it inappropriate to continue to randomise patients to one or other arm of the trial;

Recruitment is so poor that completion of the trial cannot reasonably be anticipated.

Specific Clinical criteria to stop the trial are:

Mortality

Acute bleeds

Stopping Criteria (See Appendix 2 Deliverability Plan): Since safety assessments are a key outcome, we caution against stopping the study early based on efficacy alone. Safety will focus on major bleeds (i.e. BARC (50)).

Trial management will be informed by the guideline on the 'Importance of Methodological Input to Clinical Trial Protocols' by Graham Dunn and Lisa Douet on behalf of the EME Board.

Trial management criteria to stop the trial are:

NHS contracts: All of the participating sites should have contracts in place within 6 months of the start date, in order to ensure administrative timelines are met and the trial remains within budget (Appendix 2). If sites are taking longer then this will be discussed initially with site to try and expedite approval, followed by discussion with Sponsor and Funder to determine the impact of this on the feasibility of the study.

Recruitment: All of the participating sites should have randomised patients within 3 months of the start of the initiation period (Appendix 2, Deliverability Project Plan) in order to ensure recruitment milestones are met and the trial remains within budget. If sites are taking longer than this will be discussed initially with site to discover the issue and if possible resolve this, followed by discussion with Sponsor and Funder to determine the impact of this on the feasibility of the study.

Based on agreement with the funder and sponsor, a hospital could be replaced with another if there are delays with NHS management approval and/or contract or recruitment is less than 40 randomised patients per year.

8. DATA HANDLING

8.1 Randomisation

The Robertson Centre for Biostatistics will design the eCRF, set up the study databases, and the randomisation sequence and unblinding system.

RANDOMISATION: Randomisation will be stratified by study site and location of the myocardial infarction (anterior STEMI vs. non-anterior STEMI) based on randomised permuted blocks. The randomisation system will indicate the IMP kit to be used in the catheter laboratory.

8.2 Case Report Forms / Electronic Data Record

An electronic case report form (eCRF) will be used to collect study data. The eCRF will be developed by the study Data Centre at the Robertson Centre for Biostatistics, University of Glasgow and access to the eCRF will be restricted, with only authorised site-specific personnel able to make entries or amendments to their patients' data. It is the investigator's responsibility to ensure completion and to review and approve all data captured in the eCRF.

All data handling procedures will be detailed in a Study Specific Data Management Plan. Data will be validated at the point of entry into the eCRF and at regular intervals during the study. Data discrepancies will be flagged to the study site and any data changes will be recorded in order to maintain a complete audit trail (reason for change, date change made, who made change).

8.3 Data Retention

To enable evaluations and/or audits from regulatory authorities, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records), all original signed informed consent forms, serious adverse event forms, source documents, and detailed records of treatment disposition in accordance with ICH GCP, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. Data will be retained at the Data Centre for a minimum of 5 years.

9. TRIAL MANAGEMENT

9.1 Routine management of the trial: Trial Management Group

The trial will be coordinated by the Trial Management Group. The Trial Management Group will include those individuals responsible for the day-to-day management of the trial in each site, including the Chief Investigator, Project Manager, pharmacist, and others as considered appropriate. The role of this group is to facilitate the progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The Project Manager will be employed by the Sponsor and based in the Golden Jubilee National Hospital, and will have a key role for Site Initiation in each hospital, for the on-going project coordination in each hospital (including timely delivery of trial milestones, especially recruitment) and liaising with the Sponsor.

9.2 Trial steering committee (TSC)

The role of the TSC is to provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of GCP and the relevant regulations. The TSC will make recommendations to the Sponsor regarding the continuation or termination of the trial, and in relation to protocol amendments

The Trial Steering Committee (TSC) will include:

- An independent Chair (Keith Fox)
- The Chief Investigator (Colin Berry)
- An independent Cardiologist (Raj Kharbanda, Catherine Labinjoh)
- The trial statistician
- The Project Manager
- A lay representative
- A Sponsor representative

The TSC will endeavour to meet face-to-face or by teleconference on a 4 weekly basis initially (for first 6 months) and thereafter as deemed appropriate till the end of the study.

9.3 Independent Data Monitoring Committee (IDMC)

The membership of the IDMC will be decided by the Sponsor with input from the funder. The Sponsor will put in place an IDMC Charter and the committee will determine at the first

meeting the information regarding the trial that it will require to see and the frequency of meetings.

The role of the T-TIME IDMC will be to review the accruing trial data and to assess whether there are any safety issues that should be brought to the Sponsor / Chief Investigator / TSC attention, and make recommendations on whether the trial should continue. The T-TIME IDMC will be independent of both the investigators and the funder/sponsor and will be the only body that has access to unblinded data. Ideally, the IDMC should include members with expertise in clinical trials with drugs that affect the coagulation system (e.g. thrombolysis, anti-coagulants) cardiology and/or pharmacology.

The Robertson Centre for Biostatistics at the University of Glasgow (RCB) will coordinate the data management for the trial and will facilitate reporting to the IDMC. To address risks with alteplase, the IDMC will receive monthly reports on safety data, particularly related to bleeding and stroke in addition to the planned interim analysis described in Section 4 above. The IDMC has responsibility for potentially recommending early discontinuation of the entire study or an individual arm because of safety concerns or due to futility. However on the recommendation of the funder NIHR-EME following programme/peer review, it was agreed that after approximately 40% of patients have been randomised and followed-up for 3 months, there will be a futility analysis in addition to routine safety analysis. Each active treatment arm will be compared to the placebo arm and if the conditional power for showing a benefit over placebo based on the current trend is less than 30%, then a recommendation will be made halt that arm.

9.4 *Clinical endpoints committee*

Clinical events identified as potentially relevant to the designated secondary health outcomes will be assessed by a Clinical Event Committee (CEC). The CEC will be independent of both the investigators and the funder/sponsor and will be blinded regarding any information relating to the randomisation group. The composition of the CEC will be determined by agreement with the funder and sponsor.

10. STUDY MONITORING AND AUDITING

Study Monitoring Visits will be conducted by NHS Greater Glasgow and Clyde Clinical Trials Monitor(s). Prior to commencement of the trial, a Monitoring Plan based upon the Sponsor Risk Assessment, detailing the level and type of monitoring, will be written by the Clinical Trials Monitors and approved by the Sponsor Research Governance Manager.

11. PROTOCOL AMENDMENTS

Any change in the study protocol will require an amendment. Any proposed protocol amendments will be initiated by the CI following discussion with the Sponsor and TSC and any required amendment forms will be submitted to the regulatory authority, ethics committee and sponsor. The Sponsor will determine whether an amendment is non-substantial or substantial. All amended versions of the protocol will be signed by the CI and Sponsor representative. Following a major amendment, favourable opinion/approval must be sought from the original reviewing REC, MHRA (where appropriate) and Research and Development (R&D) office. The Chief Investigator will be responsible for informing the Trial Management Group of all protocol amendments.

12. ETHICAL CONSIDERATIONS

12.1 Ethical conduct of the study

The study will be carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and its revisions (Tokyo [1975], Venice [1983], Hong Kong [1989], South Africa [1996], Edinburgh [2000], Seoul [2008] and Fortaleza [2013]).

Favourable ethical opinion will be sought from West of Scotland Research Ethics Committee before patients are entered into this clinical trial. Patients will only be allowed to enter the study once they have provided witnessed verbal informed consent (written informed consent to be sought at a later time as per Section 3.5.1).

The Chief Investigator and/or Sponsor will be responsible for updating the Ethics committee of any new information related to the study.

12.2 Informed consent (verbal and written)

During the emergency care procedure, witnessed verbal informed consent will initially be obtained in the cardiac catheterisation laboratory (3.5.1.1). Written informed consent will subsequently be obtained from each trial participant when on the ward, usually later that day or the next day (3.5.1.2). The Clinical Research Nurse or clinical investigator will explain the exact nature of the study in writing, provision of patient information sheet, and verbally. This will include the known side-effects that may be experienced, and the risks of participating in this clinical trial. Trial participants will be informed that they are free to withdraw their consent from the study or study treatment at any time.

13. INSURANCE AND INDEMNITY

The T-TIME trial is co-sponsored by NHS Greater Glasgow & Clyde and The University of Glasgow. The sponsors will be liable for negligent and non-negligent harm associated with the trial. NHS indemnity is provided under the Clinical Negligence and Other Risks Indemnity Scheme (CNORIS) in Scotland, and the equivalent scheme in England. As the substantive employer of the CI and as co-sponsor of the T-TIME trial, The University of Glasgow also has clinical trials insurance. It will be confirmed prior to the trial starting that insurance cover will be provided automatically under the current policy. The insurance cover will be subject to the appropriate authorisations being received from the MHRA, Ethics, and the NHS.

The NHS has a duty of care to patients treated, whether or not the patient is taking part in a clinical trial, and the NHS remains liable for clinical negligence and other negligent harm to patients under its duty of care.

As this is a clinician-led study there are no arrangements for no-fault compensation.

14. FUNDING, PEER REVIEW AND PUBLIC INVOLVEMENT

14.1 Funding and peer review

T-TIME was initially approved as a single centre trial in 180 patients (Medical Research Council (MRC) / National Institute for Health Research (NIHR) – Efficacy and Mechanism Evaluation Programme (EME) grant (November 2012; reference 10/90/12) and full ethics approval was granted (REC 12/WS/0109, EudraCT number 2012-001123-13). Following publication of the INFUSE-AMI trial (20), the EME Board recommended a larger trial would be needed to enhance clinical impact. The current study design has been developed through peer review with external reviewers and the NIHR-EME Board panel of experts and Programme Management.

14.2 Patient and public involvement

Public contributors reviewing the study design including Mr Ken Timmis of the Heart Care Partnership (a patient forum associated with the British Cardiovascular Society), Mr David Craig, Lay Advisor to the Golden Jubilee National Hospital and Dr Gordon Baird, retired General Practitioner from Stranraer, Dumfries and Galloway. A Lay Advisor will be included in the Trial Steering Committee as agreed with the EME Programme Management.

15. CO-SPONSOR RESPONSIBILITIES

NHS Greater Glasgow & Clyde and the University of Glasgow

Prior to study initiation, a non-commercially funded clinical trial co-sponsorship agreement will be put in place between NHS Greater Glasgow & Clyde and The University of Glasgow. The roles and liabilities each organisation will take under The Medicines for Human Use (Clinical Trials) Regulations, 2004 SI 2001:1031 are laid out in this agreement signed by both organisations. The University of Glasgow shall be responsible for carrying out the obligations and responsibilities set out in the aforementioned agreement, and shall be deemed "sponsor" for the purposes of, Part 3 of the regulations in relation to the study. NHS Greater Glasgow & Clyde shall be responsible for carrying out the responsibilities set out in the agreement, and shall be deemed "sponsor" for the purposes of, Parts 4, 5, 6 and 7 of the Regulations in relation to the study.

16. ANNUAL REPORTS

A biannual progress report will be submitted to the funder, the first being submitted 6 months from the date that all trial related approvals are in place. Annual reports will be submitted to the ethics committee, regulatory authority and sponsor with the first submitted one year after the date that all trial related approvals are in place.

17. DISSEMINATION OF FINDINGS

The study will be assigned an International Standard Randomised Controlled Trial Number (ISRCTN) and will be registered on the clinicaltrials.gov website before participant recruitment commences.

It is anticipated that the results will be published in a peer reviewed journal. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the Co-Sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study Co-Sponsor. Any investigator involved with this study is obligated to provide the Co-Sponsor with complete test results and all data derived from the study.

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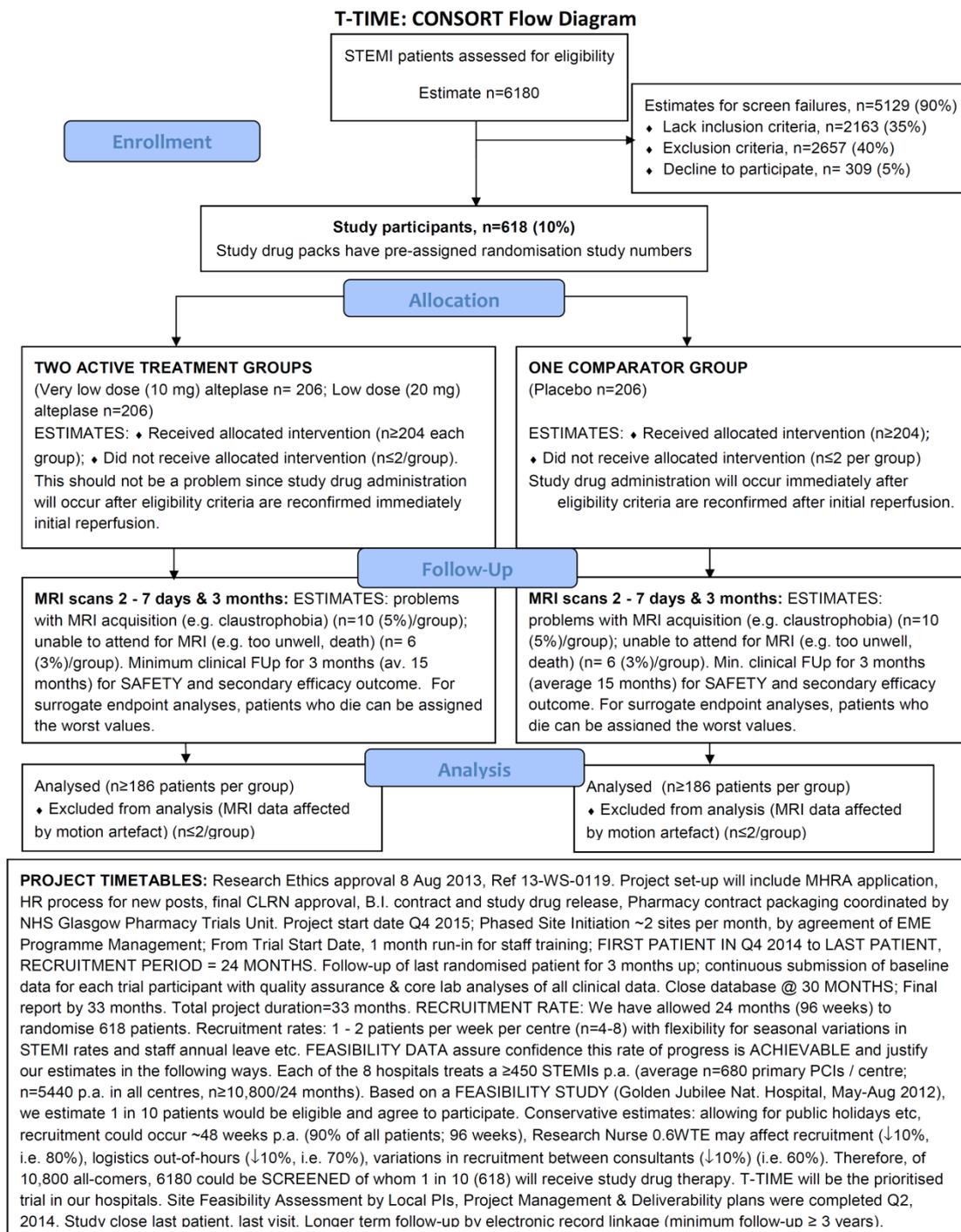
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Appendix 1

Figure - Flow diagram of the clinical trial



Appendix 2

Deliverability Project Plan with Stop/Go criteria

Chief Investigator	Colin Berry
Project ref number	MRC-NIHR-EME 12-170-45
Project title	A randomised parallel group double blind placebo-controlled dose ranging Trial of low dose adjunctive alTeplase during priMary PCI (T-TIME).

- Stop/Go decision points will form part of the contract with the Department of Health, and will be used by the EME Programme to review whether funding should continue at each identified stage of the project.
- Stop/Go points will be used if there is a realistic expectation that there could be good reasons (other than failure to deliver) for a project not to continue to the next stage.
- The tables identify the stages of the project and the criteria used in deciding whether the project should progress.

Stop/Go Decision Point 1 (S/G 1)

Time from start to S/G 1 (months)	6 months
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Milestones for S/G 1 (max 250 words, bulleted)
<ul style="list-style-type: none">➤ The first patient first visit will be achieved 6 months from the start date.➤ By 12 months from the contracted start date all sites should have been 'opened' / initiated.

Success criteria and target values (max 250 words, bulleted)
<ul style="list-style-type: none">➤ SUCCESS CRITERIA: All sites opened within 6 months of the Sponsor approval letter➤ STOP CRITERIA: <50% of sites without initiation within 6 months of the Sponsor approval letter.➤ If <30% of predicted 6 month target of participants have been randomised by 6 months after the start date for recruitment then the process should be reviewed

with a view to improvement. If deemed by the Sponsor unable to remedy then decision to be taken by Sponsor in conjunction with TSC as to viability of project.

Reports required during S/G 1

- Sponsor and Trial Management Group report on R&D site approvals

Stop/Go Decision Point 2 (S/G 2)

Time from start to S/G 2 (months)

Anticipated month 6

Milestones for S/G 2 (max 250 words, bulleted)

- Safety analysis after 10% of the study participants have been randomised.

Success criteria and target values (max 250 words, bulleted)

- The iDMC will establish the safety criteria for stopping on review of the first 10% of patients. Major bleeds will most likely be the outcome and the incidence threshold for stopping and will be decided by the iDMC before the trial starts and defined in their charter.

Reports required during S/G 2

Trials Unit report on adverse events

Stop/Go Decision Point 3 (S/G 3)

Time from start to S/G 3 (months)

15 months

Milestones for S/G 3 (max 250 words, bulleted)

- Futility analysis after 40% of patients have been randomised and followed-up for 3 months.
- IDMC report on the futility analysis
-

Success criteria and target values (max 250 words, bulleted)

Each active treatment arm will be compared to the placebo arm and if the conditional power for showing a benefit over placebo based on the current trend is less than 30%, then a recommendation will be made by the iDMC to halt that arm.

The IDMC report on the futility analysis will be a S/G criteria.

Reports required during S/G 3

Trial Management Group report on recruitment.

