SUPPLEMENTAL METHODS & RESULTS

Effect of coronary flow on intracoronary alteplase, a pre-specified analysis from a randomised trial

ClinicalTrials.gov: NCT02257294

Contents

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Eligibility Criteria

Patients with a clinical diagnosis of acute ST-segment elevation myocardial infarction (STEMI) were eligible for randomisation according to the following eligibility criteria:

Inclusion

- Acute MI (symptom onset ≤ 6 hours) with persistent ST-segment elevation or recent left bundle branch block
- Coronary artery occlusion (TIMI [Thrombolysis in Myocardial Infarction] coronary flow grade 0 or 1), or impaired coronary flow (TIMI coronary flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2 or more)
- Proximal-mid culprit lesion location in a major coronary artery (i.e. the right, left anterior descending, intermediate, or circumflex artery)
- Radial artery access
- Successful coronary reperfusion (TIMI coronary flow grade ≥2) pre-stent achieved prior to randomisation.
- Informed consent, i.e. only patients who were sufficiently well to understand the information about the study, as described by the attending cardiologist, were eligible to participate.

Exclusion

- 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)
- Cardiogenic shock (Killip Class IV)
- Multivessel percutaneous coronary intervention (PCI) intended before the day 2-7 cardiovascular magnetic resonance (CMR) scan
- Estimated body weight <60 kg

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- Non-cardiac co-morbidity with expected survival <1 year
- Contra-indication to contrast-enhance CMR imaging
- Pacemaker, or implantable defibrillator
- Known impaired renal function (estimated glomerular filtration rate <30ml/min)
- Significant bleeding disorder either at present or within the past 6 months
- Known haemorrhagic diathesis
- Patient with current concomitant oral anticoagulation therapy (international normalised ratio >1.3), including apixaban, dabigatran and rivaroxaban
- Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
- Severe hypertension (blood pressure >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 acute MI)
- Recent head trauma (<2 months)
- Prolonged cardiopulmonary resuscitation (>2 minutes) within the past 2 weeks
- Acute pericarditis and/ or subacute bacterial endocarditis
- 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 ischemic attack in the preceding 6 months
- Dementia

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- Hypersensitivity to gentamicin, or natural rubber
- Incapacity, or inability to provide informed consent
- Previous randomisation 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-menopausal), or breast feeding
- Requirement for immunosuppressive therapy at any time during the preceding 3 months. This would include corticosteroids (but not inhaled or topical), drugs used following transplantation (e.g tacrolimus, cyclosporine), anti-metabolite therapies (e.g. mycophenolic acid, azathioprine, leflunomide and immunomodulators including biologics (e.g. adalimumab, or etanercept) and disease modifying anti-rheumatic drugs. This list is not exhaustive.
- 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 preceding 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.
- Any significant concurrent, or recent condition(s) not listed above that in the opinion of the treating clinician would pose an additional risk to the patient.

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Standard Care

Standard care for coronary reperfusion was with balloon angioplasty, or aspiration thrombectomy for thrombus-containing lesions. A coronary balloon diameter (mm) vs. lumen diameter (mm) relationship of <1:1 and a low inflation pressure were recommended to minimise thrombus embolization. The balloon angioplasty was intended to stabilise the thrombotic lesion and prevent vessel re-occlusion prior to stent implantation. Anti-thrombotic therapy included oral anti-platelet drugs and intravenous heparin (5000 IU, or as per standard practice) at the first medical contact. The target activated clotting time (ACT) was 250s.

Interventions

After initial balloon angioplasty/ thrombus aspiration, the participants were randomised using an interactive voice response-based system, and then received the allocated intervention. The study drug (placebo, alteplase 10mg, or alteplase 20mg) was manually infused before stent implantation. The drug was reconstituted by the clinical staff using 20ml of sterile water for injection. The cardiologist then infused the solubilised drug over 5-10 minutes directly into the culprit artery, proximal to the culprit lesions, using either an intracoronary catheter or the guiding catheter if selectively engaged.

Angiogram Acquisition & Analysis Methods

Coronary angiograms were acquired during emergency care with cardiac catheter laboratory X-ray and information technology equipment. The angiograms were analysed using post-processing software (QAngio® XA Medis, Leiden, NL.) by experienced investigators who were blinded to treatment allocation. Catheter calibration was performed using the catheter calibration function on MEDIS QAngio. For each lesion, a view perpendicular to the long axis of the vessel was used in order to avoid foreshortening and overlap of branches. The single plane projection showing the best opacified and most severe lesion with minimal foreshortening and minimal branch overlap was selected. Feedback was provided to sites on the quality and completeness of the angiograms.

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TIMI Coronary Flow Grade

The TIMI coronary flow grade was assessed using the following definitions(1):

TIMI coronary flow grade	Definition
0	No flow
1	Minimal flow past obstruction
2	Slow (but complete) filling and slow clearance
3	Normal flow and clearance

TIMI Myocardial Perfusion Grade

TIMI myocardial perfusion grade provides a score for ground-glass appearance ('blush') of the contrast entering the microvasculature and contrast washout. TIMI myocardial perfusion grade was assessed according to the following definitions(2):

TIMI myocardial perfusion grade	Definition
0	Minimal or no myocardial blush in the distribution of the culprit artery.
1	Myocardial blush is present in the distribution of the culprit artery. But there is incomplete clearance of dye between injections (with ~ 30 seconds between injections).
2	Myocardial blush is present in the distribution of the culprit artery. But there is slow contrast entry into the microvasculature and slow clearance of contrast. Specifically, blush is strongly persistent (i.e. either does not or only minimally diminishes in intensity) beyond 3 cardiac cycles after injection.
3	Myocardial blush is present in the distribution of the culprit artery, with normal entry and exit of dye (mild/ moderate persistence of dye beyond 3 cardiac cycles, but notably reduced after 3 cardiac cycles). Blush that is only mild intensity throughout 3 cardiac cycles after injection (washout phase), but fades minimally is also classified as grade 3.

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116 ***TIMI Frame Count***

117 The TIMI frame count represents the amount of time (in frames) for contrast dye to reach
118 a standardized distal landmark.(2) If the culprit vessel was the left anterior descending artery
119 the frame count was divided by 1.7 (correcting for longer vessel length).

120 ***TIMI Coronary Thrombus Grade***

121 Thrombus burden revealed during coronary angiography was classified according to the
122 TIMI thrombus grade(3):

Thrombus grade	Definition
0	No angiographic characteristics of thrombus are present
1	Possible thrombus is present, with 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 ≤ half 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

123

124 ***Lesion Characterisation***

125 The culprit lesions were assessed for complexity using the modified American College of
126 Cardiology/ American Heart Association score, which characterises coronary lesions as type
127 A, B1 (one characteristic of a type B lesion), B2 (two or more characteristics of a type B
128 lesion) and C.(4)

129 The culprit lesions were also assessed for complexity using a 6-point plaque
130 characterisation score,(5) comprising:

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(i) Intraluminal filling defect consistent with thrombus

(ii) Ulcerated appearance, for example hazy contour, and/ or apple-core appearance

(iii) Irregularity of vessel borders

(iv) TIMI flow <3 beyond the lesion

(v) Moderate to severe calcification, i.e. calcification in more than one cine, outlining the full lumen

(vi) Lesion at a bifurcation point

CMR Acquisition and Analysis

CMR was performed using 1.5-T platforms (Siemens MAGNETOM Avanto, Erlangen, Germany and Philips Intera, Best, The Netherlands). The imaging protocol followed a standard operating procedure that included planning and localisers, T1-mapping, T2*-mapping, cine CMR with steady-state free precession (SSFP), and late gadolinium enhancement imaging 10 – 15 minutes after administration of contrast media.(6) The scan acquisitions were spatially co-registered and also included different slice orientations to enhance diagnostic confidence.

The intravenous contrast agent used in this study was gadobutrol (Gadovist®, Bayer: 1.5 mmol/ml solution for injection), which was administered in two doses. The first dose injection (0.05 mmol/kg) was given to initiate the first-pass of contrast. The second dose (0.1 mmol/kg) was given immediately after the first-pass. Therefore, the total dose of gadobutrol was 0.15 mmol/kg.

SSFP cine breath-hold sequences (with parallel imaging acceleration) were used. The heart was imaged in multiple parallel SAX planes 8-mm thick, separated by 2mm gaps, equating to approximately 10 slices and 30 cardiac phases. The CMR analyses were undertaken using Medis® Suite MR (Medis, Leiden, NL), by two trained investigators who were blinded to treatment allocation.

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Late Gadolinium Enhancement

Late microvascular obstruction (MVO) was imaged 10-15 minutes after intravenous Gadovist contrast administration, using in general a motion corrected T1-weighted phase-sensitive inversion recovery radiofrequency pulse sequence. A full stack, aligned to T2* scans (or cines) and 3 long axis views (vertical long axis, horizontal long axis and 3 chamber view) were acquired.

MVO was defined as a dark zone on early gadolinium enhancement imaging 1, 3, 5 and 7-minutes post-contrast injection that remained present within an area of late gadolinium enhancement at 15 minutes. The endocardial and epicardial borders were contoured. The myocardial mass (grams) of the dark zone was quantified by manual delineation and expressed as a percentage of total left ventricular (LV) mass.

Infarct Size

The presence of acute infarction was established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and late gadolinium enhancement imaging in two imaging planes. The myocardial mass of late gadolinium (grams) was quantified using computer assisted planimetry and the territory of infarction was delineated using a 5 standard deviation method and expressed as a percentage of total LV mass. Typical late gadolinium enhancement and MVO imaging parameters with phase sensitive inversion recovery: matrix 192 x 256 pixels; flip angle 25º; TE 3.36 ms; bandwidth 130 Hz/pixel; echo spacing 8.7ms and trigger pulse 2. The voxel size is 1.8 x 1.3 x 8 mm. Inversion times individually adjusted to optimize nulling of apparently normal myocardium (typical values, 200 to 300ms).

Myocardial Oedema

The presence of myocardial oedema was established based on an area of increased signal intensity on the SSFP cine images (acquired two minutes after gadolinium contrast

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injection). The myocardial mass was calculated by manual delineation in end-diastole and end-systole. The values were averaged and expressed as a percentage of LV mass.(6)

Myocardial Salvage

Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at risk, as reflected by the extent of oedema. The myocardial salvage index was calculated by dividing the myocardial salvage area by the initial area-at-risk.

Myocardial Haemorrhage

On the T2* parametric maps, a threshold of 20ms was applied. A region of reduced signal intensity within the infarcted area, with a T2* value of <20 ms(7)(8) was considered to confirm the presence of myocardial haemorrhage. The area was manually delineated and expressed as % LV mass.

Local Hospital Blood Sample Handling

Blood samples were measured when site logistics permitted. The sampling time-points were 0, 2 and 24 hours post-PCI. Blood samples were collected into 0.109M sodium citrate (for haemostasis assays), or EDTA (Troponin). The blood samples were centrifuged locally and plasma separated and frozen within 2 hours of sampling. Frozen plasma samples were subsequently transported on dry ice for central laboratory analysis in the department of Haematology, Macewan Building, 16 Alexandra Parade, Glasgow Royal Infirmary, G31 2ER. Plasma samples were stored at -80ºC until analysis, with residual samples being transferred to the Glasgow Biorepository for storage at the end of the study.

Central Laboratory Analysis for Troponin T

EDTA plasma samples were stored at -80ºC in the Glasgow Royal Infirmary until batch analysis at the end of the study. The biochemical analyses were performed in the British Heart Foundation Glasgow Cardiovascular Research Centre.

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EDTA plasma samples were stored to analyse high-sensitivity cardiac troponin T (ng/ml) on first thaw. Serial measurements of troponin T using the Roche high-sensitivity assay were used to provide a biochemical measurement of infarct size (area-under-the-curve).

For measurement of high sensitivity cardiac troponin T, we used an automated method (e411, Roche Diagnostic, Burgess Hill, U.K.) calibrated and quality controlled using the manufacturers reagents. We also participated in the National External Quality Assurance Scheme (NEQAS). The lower limit of detection of Troponin T is 0.003 ng/ml and the 99th percentile value in a healthy subpopulation is 0.0014 ng/ml (Roche Diagnostics, data on file). The between-assay coefficient of variations were 2.2% and 4.2% for control materials with mean Troponin T concentrations of 2.098 ng/ml and 0.00027 ng/ml, respectively.

Central Laboratory Analysis for Coagulation Parameters

The coagulation parameters measured in this study included fibrinogen and plasminogen (both measures of coagulation and systemic fibrinolysis), fibrin D-Dimer (a measure of fibrin lysis), tissue plasminogen activator (tPA) (a measure of endogenous tPA and any circulating alteplase) and prothrombin fragment F1+2 (a measure of thrombin activation). A depletion of fibrinogen and plasminogen following thrombolysis correlates with systemic fibrinolysis and may correlate with bleeding risk. Prothrombin fragment F1+2 is a measure of thrombin activation and correlate with the (undesired) procoagulant effect of thrombolysis. Prothrombin fragment F1+2 is depressed by anti-coagulants administered before and during PCI.

Standard laboratory assays (Fibrinogen by Clauss method; high sensitivity Fibrin D-Dimer by latex immunoassay; and Plasminogen Activity by chromogenic assay were performed on an IL TOP700 analyser using HemosIL® reagents (Instrumentation Laboratory Company, Bedford, U.S.). The fibrinogen Clauss assay had a normal reference rages 170 – 4.0 g/L (internally derived) and an inter-assay coefficient of variation of 5.8% and 7.7% for

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low control samples with mean concentrations of 2.92 g/L and 2.22 g/L respectively. The fibrin D-Dimer assay had a normal reference range <0.230 μg/ml (manufacturer derived), and an inter-assay coefficient of variation of 11.7% and 5.2% for control samples with mean concentrations of 0.343 μg/ml and 0.770 μg/ml respectively. The plasminogen activity assay had a normal reference rage 80 – 133 U/dL (manufacturer derived), and an inter-assay coefficient of variation of 2.1% and 1.8% for control samples with mean concentrations of 95.4 U/dL and 29.6 U/dL, respectively.

Non-standard laboratory ELISA assays (tissue plasminogen activator [tPA] and Prothrombin fragment F1+2 antigen levels) were performed on a TECAN Sunrise spectrophotometer (Labtech International Ltd, U.K.) using Zymutest tPA Antigen (Hyphen BioMed, Neuville-sur-oise France) and Enzygnost F1+2 Mono (Siemens, Marburg, Germany) commercial kits respectively. The tPA antigen assay had a normal reference range <10 ng/ml (manufacturer derived), and an inter-assay coefficient of variation of 4.7% and 11% for control samples with mean concentrations of 11.0 ng/ml and 3.1 ng/ml, respectively. The F1+2 assay had a normal reference rage 69 – 229 pmol/L (manufacturer derived) and an inter-assay coefficient of variation of 7.9% for a normal control sample with a mean concentration of 97.6 pmol/L.

Trial Management

There was a Trial Management Group for operational activity, an independent Data and Safety Monitoring Committee and a Trial Steering Committee to coordinate the trial and liaise with the Sponsor and Trials Unit. Each committee had a charter that was established before enrolment started.

The independent Data and Safety Monitoring Committee met before the enrolment began, and twice again during the active phase of the trial. This committee had responsibility for potentially recommending early discontinuation of the entire study or an individual arm,

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because of safety concerns or due to futility. The funder, the Efficacy and Mechanism Evaluation (EME) program of the National Institute for Health Research (NIHR) required an interim analysis for futility and also specified the criteria. Following a prespecified futility analysis, performed when 40% of the trial population had reached 3 months follow-up, the Data and Safety Monitoring Committee recommended that enrolment into the T-TIME trial should be discontinued on December 21 2017.

The Robertson Centre for biostatistics within the Glasgow Clinical Trials Unit provided the trial-specific electronic data collection system, acted as an independent coordination centre for randomisation and data management. The trial was approved by the National Research Ethics Service (reference 13/WS/0119). The clinical trial registration number is NCT02257294 and the trial was co-sponsored by the University of Glasgow and greater Glasgow and Clyde Health Board, NHS Scotland. The sponsor undertook feasibility assessments at each site, visits were undertaken in all of the sites. All serious adverse events were prospectively reported to the Pharmacovigilance Unit.

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Supplemental Table 1. Additional procedure characteristics, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%).

	Impaired coronary flow (TIMI flow ≤ 2)				Normal coronary flow (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
American Heart Association culprit lesion type: *								
B2	42 (27%)	13 (26%)	15 (31%)	14 (26%)	62 (23%)	17 (19%)	20 (23%)	25 (28%)
C	112 (73%)	37 (74%)	34 (69%)	41 (75%)	205 (77%)	75 (82%)	67 (77%)	63 (72%)
Culprit lesion plaque characterisation score: † *								
2	1 (1%)	0	1 (2%)	0	3 (1%)	1 (1%)	2 (2%)	0
3	20 (13%)	5 (10%)	6 (12%)	9 (16%)	76 (29%)	27 (29%)	26 (30%)	23 (26%)
4	115 (75%)	40 (80%)	35 (71%)	40 (73%)	164 (61%)	54 (59%)	53 (61%)	57 (65%)
5	17 (11%)	4 (8%)	7 (14%)	6 (11%)	24 (9%)	10 (11%)	6 (7%)	8 (9%)
6	1 (1%)	1 (2%)	0	0	0	0	0	0
QCA lesion length pre-drug (mm) *	25.5 ± 11.2	26.7 ± 11.6	27.4 ± 12.4	22.7 ± 9.3	27.2 ± 11.3	26.7 ± 10.6	27.6 ± 11.6	27.5 ± 11.8
Total number of stents deployed:								
0	2 (1%)	0	1 (2%)	1 (2%)	1 (0.0%)	1 (1%)	0	0
1	104 (68%)	35 (70%)	29 (59%)	40 (73%)	188 (70%)	59 (64%)	65 (75%)	64 (73%)
2	40 (26%)	13 (26%)	14 (29%)	13 (24%)	64 (24%)	30 (3%)	14 (16%)	20 (23%)
≥3	8 (5%)	2 (4%)	5 (10%)	1 (2%)	14 (5%)	2 (2%)	8 (9%)	4 (5%)

***** The angiographic parameters are based on central laboratory assessments.

† The plaque characterisation score comprised one point for each of: intraluminal thrombus, ulceration, irregularity of vessel borders, TIMI flow <3 beyond the lesion, moderate-severe calcification and bifurcation.

Abbreviations: QCA, quantitative coronary angiography.

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Supplemental Table 2. Analysis of CMR derived LV end-diastolic and end-systolic volumes, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Data are median [IQR].

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)*	Alteplase 10mg (n=136)*	Alteplase 20mg (n=143)*	Alteplase 10mg vs placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
CMR parameters 2-7 days after primary PCI								
LV end-diastolic volume (ml)								
TIMI flow ≤2	174.2 [153.9, 214.1]	177.3 [163.9, 212.3]	161.6 [142.6, 200.0]	1.02 (0.93, 1.12) p=0.721	0.94 (0.86, 1.03) p=0.171	0.340	0.97 (0.90, 1.06) p=0.525	0.141
TIMI 3 flow	162.2 [141.8, 190.1]	176.5 [155.5, 205.8]	170.5 [136.6, 194.3]	1.08 (1.01, 1.16) **p=0.021**	1.02 (0.95, 1.09) p=0.601		1.05 (0.99, 1.11) p=0.105	
LV end-systolic volume (ml)								
TIMI flow ≤2	96.2 [80.2, 118.9]	105.3 [85.6, 124.3]	95.5 [80.8, 113.6]	0.0 (-0.85, 0.86) p=0.993	0.73 (-0.08, 1.54) p=0.080	0.261	0.40 (-0.32, 1.13) p=0.277	0.284
TIMI 3 flow	90.2 [75.9, 108.0]	92.9 [79.0, 113.5]	92.5 [72.3, 109.0]	-0.12 (-0.80, 0.57) p=0.738	-0.10 (-0.80, 0.60) p=0.778		-0.11 (-0.70, 0.49) p=0.72	
CMR parameters 3 months after primary PCI								
LV end-diastolic volume (ml)								
TIMI flow ≤2	170.2 [158.8, 207.1]	170.0 [152.9, 206.4]	174.0 [150.5, 195.1]	1.01 (0.91, 1.12) p=0.796	0.95 (0.86, 1.05) p=0.349	0.567	0.98 (0.90, 1.07) p=0.673	0.281
TIMI 3 flow	157.9 [138.9, 188.5]	173.6 [153.7, 205.6]	162.9 [140.4, 194.3]	1.08 (1.00, 1.16) **p=0.045**	1.01 (0.94, 1.08) p=0.847		1.04 (0.98, 1.11) p=0.213	
LV end-systolic volume (ml)								
TIMI flow ≤2	81.6 [72.8, 114.7]	88.9 [71.4, 116.5]	92.1 [71.5, 110.1]	1.03 (0.88, 1.20) p=0.729	0.99 (0.85, 1.15) p=0.878	0.762	1.01 (0.88, 1.15) p=0.923	0.508
TIMI 3 flow	77.5 [60.7, 99.5]	85.9 [71.7, 103.3]	78.5 [65.8, 102.1]	1.10 (0.99, 1.23) p=0.085	1.03 (0.92, 1.15) p=0.640		1.06 (0.97, 1.17) p=0.210	

Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

* Missing data: LV volumes 2 – 7 days after primary PCI (n=34), LV volumes 3 months after primary PCI (n=63).

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Supplemental Table 3. Analysis of selected CMR parameters 2-7 days after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration, and by subgroups of MI location (anterior [n=187], non-anterior [n=234]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, or n (%), unless otherwise stated.

		Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
		Placebo (n=142)*	Alteplase 10mg (n=136)*	Alteplase 20mg (n=143)*	Alteplase 10mg vs placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
					Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
MVO presence (n/ total) (a)									
Anterior-MI:	TIMI flow ≤2	8/23 (34.8)	11/22 (50.0)	15/27 (55.6)	1.88 (0.57, 6.21) p=0.304	2.34 (0.75, 7.37) p=0.145	0.354	2.12 (0.77, 6.13) p=0.151	0.150
	TIMI 3 flow	19/36 (52.8)	16/34 (47.1)	15/31 (48.4)	0.80 (0.31, 2.03) p=0.633	0.84 (0.32, 2.19) p=0.720		0.82 (0.36, 1.84) p=0.625	
Non-anterior MI:	TIMI flow ≤2	7/21 (33.3)	14/20 (70.0)	11/22 (50.0)	4.67 (1.25, 17.44) **p=0.022**	2.00 (0.58, 6.87) p=0.271	**0.014**	2.94 (0.98, 8.81) p=0.054	**0.012**
	TIMI 3 flow	22/49 (44.9)	13/46 (28.3)	18/52 (34.6)	0.48 (0.21, 1.14) p=0.095	0.65 (0.29, 1.45) p=0.292		0.57 (0.28, 1.15) p=0.116	
MVO extent (% LV mass)† (b)									
Anterior-MI:	TIMI flow ≤2	3.7 ± 7.4	2.5 ± 3.4	6.3 ± 8.0	0.0 (-0.85, 0.86) p=0.993	0.73 (-0.08, 1.54) p=0.080	0.261	0.40 (-0.32, 1.13) p=0.277	0.284
	TIMI 3 flow	3.0 ± 4.1	2.9 ± 4.6	3.1 ± 5.6	-0.12 (-0.80, 0.57) p=0.738	-0.10 (-0.80, 0.60) p=0.778		-0.11 (-0.70, 0.49) p=0.72	
Non-anterior MI:	TIMI flow ≤2	1.4 ± 2.9	3.1 ± 4.5	4.2 ± 6.5	0.65 (-0.07, 1.37) p=0.079	0.71 (0.00, 1.41) p=0.050	0.156	0.68 (0.07, 1.29) p=0.031	0.053
	TIMI 3 flow	1.5 ± 2.6	2.1 ± 4.9	1.8 ± 3.3	-0.07 (-0.54, 0.40) p=0.775	-0.02 (-0.48, 0.44) p=0.922		-0.04 (-0.45, 0.36) p=0.828	
Myocardial haemorrhage presence (n/ total) (a)									
Anterior-MI:	TIMI flow ≤2	7/21 (33.3)	10/19 (52.6)	15/27 (55.6)	2.22 (0.62, 7.98) p=0.221	2.50 (0.77, 8.16) p=0.129	0.245	2.38 (0.83, 7.32) p=0.114	0.102
	TIMI 3 flow	17/34 (50.0)	15/33 (45.5)	12/29 (41.4)	0.83 (0.32, 2.18) p=0.710	0.71 (0.26, 1.92) p=0.494		0.77 (0.33, 1.79) p=0.544	
Non-anterior MI:	TIMI flow ≤2	4/19 (21.1)	12/19 (63.2)	11/22 (50.0)	6.43 (1.62, 30.35) **p=0.012**	3.75(0.99, 16.56) p=0.061	**0.007**	4.79 (1.45, 19.13) **p=0.015**	**0.003**
	TIMI 3 flow	22/48 (45.8)	13/43 (30.2)	18/52 (34.6)	0.51 (0.22, 1.22) p=0.129	0.63 (0.28, 1.40) p=0.254		0.57 (0.28, 1.17) p=0.124	
Myocardial haemorrhage extent (% LV mass)† (c)									
Anterior-MI:	TIMI flow ≤2	2.9 ± 7.1	2.8 ± 4.0	4.6 ± 6.4	-0.08 (-3.22, 3.05) p=0.959	1.74 (-1.10, 4.64) p=0.230	0.472	1.01 (-1.59, 3.61) p=0.447	0.752
	TIMI 3 flow	1.6 ± 3.0	2.0 ± 3.4	2.2 ± 5.2	0.39 (-2.08, 2.87) p=0.757	0.55 (-2.01, 3.10) p=0.676		0.46 (-1.71, 2.64) p=0.677	
Non-anterior MI:	TIMI flow ≤2	0.4 ± 1.1	1.6 ± 2.5	2.9 ± 4.9	1.21 (-0.94, 3.36) p=0.272	2.52 (0.56, 4.47) **p=0.012**	0.072	1.99 (0.23, 3.75) **p=0.028**	0.067
	TIMI 3 flow	1.3 ± 2.7	1.6 ± 3.8	1.1 ± 2.8	0.28 (-1.04, 1.60) p=0.679	-0.16 (-1.43, 1.11) p=0.804		0.04 (-1.08, 1.16) p=0.944	

Infarct size (% LV mass) (c)									
Anterior-MI:	TIMI flow ≤2	33.4 ± 17.0	37.6 ± 11.5	35.3 ± 15.4	4.13 (-3.97, 12.23) p=0.319	1.91 (-5.80, 9.61) p=0.628	0.382	2.91 (-3.94, 9.75) p=0.407	0.180
	TIMI 3 flow	331. ± 12.7	31.3 ± 12.0	28.4 ± 14.5	-1.75 (-8.24, 4.75) p=0.598	-4.71 (-11.36, 1.95) p=0.167		-3.16 (-8.79, 2.47) p=0.272	
Non-anterior MI:	TIMI flow ≤2	22.3 ± 11.4	25.8 ± 11.3	28.5 ± 8.3	3.57 (-2.35, 9.49) p=0.238	6.23 (0.45, 12.01) p=0.036	0.097	4.96 (-0.08, 10.01) p=0.055	**0.045**
	TIMI 3 flow	20.8 ± 9.8	19.8 ± 8.8	19.5 ± 9.4	-1.06 (-4.95, 2.83) p=0.595	-1.34 (-5.11, 2.43) p=0.488		-1.21 (-4.51, 2.1) p=0.475	
Myocardial salvage index (c)									
Anterior-MI:	TIMI flow ≤2	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	-0.09 (-0.22, 0.04) p=0.197	-0.05 (-0.18, 0.08) p=0.434	0.217	-0.07 (-0.18, 0.04) p=0.241	0.085
	TIMI 3 flow	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.3	0.05 (-0.06, 0.15) p=0.402	0.08 (-0.03, 0.18) p=0.173		0.06 (-0.03, 0.15) p=0.202	
Non-anterior MI:	TIMI flow ≤2	0.4 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	-0.03 (-0.18, 0.12) p=0.676	-0.13 (-0.28, 0.01) p=0.077	0.213	-0.08 (-0.21, 0.04) p=0.196	0.231
	TIMI 3 flow	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.00 (-0.10, 0.10) p=0.990	0.02 (-0.08, 0.11) p=0.740		0.01 (-0.07, 0.09) p=0.836	
LV ejection fraction (%) (c)									
Anterior-MI:	TIMI flow ≤2	38.8 ± 12.6	39.8 ± 8.0	40.8 ± 8.8	0.96 (-4.10, 6.02) p=0.711	2.03 (-2.83, 6.9) p=0.414	0.969	1.54 (-2.77, 5.84) p=0.485	0.896
	TIMI 3 flow	41.0 ± 8.4	41.3 ± 7.7	43.2 ± 7.0	0.29 (-3.81, 4.39) p=0.890	2.12 (-2.08, 6.32) p=0.323		1.16 (-2.39, 4.71) p=0.522	
Non-anterior MI:	TIMI flow ≤2	48.4 ± 3.2	43.4 ± 9.5	44.7 ± 8.4	-4.97 (-9.35, -0.59) **p=0.027**	-3.69 (-7.92, 0.54) p=0.089	0.199	-4.29 (-8.00, -0.57) **p=0.025**	0.093
	TIMI 3 flow	47.4 ± 6.3	47.2 ± 6.1	46.8 ± 8.2	-0.25 (-3.10, 2.59) p=0.861	-0.69 (-3.47, 2.09) p=0.627		-0.48 (-2.91, 1.94) p=0.697	

(a) Treatment effect estimates reported as odds ratios between groups, from a logistic regression model.

(b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model.

(c) Treatment effect estimates reported as mean differences between groups, from linear regression.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

***** Missing data: MVO extent, or presence/ absence (n=38); myocardial haemorrhage extent (n=73); myocardial haemorrhage presence/ absence (n=55); infarct size, or myocardial salvage index (n=38); LV ejection fraction (n=34)

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† Given the high proportion of participants with a 0 value for MVO amount (56% of participants), and myocardial haemorrhage amount (57% of participants) the median value for MVO and myocardial haemorrhage was 0 for all groups, while the mean (SDs) are not ideal summaries for these data. It has been reported as such for this reason.

Abbreviations: CI, confidence interval; CMR, cardiovascular magnetic resonance; LV, left ventricular; MI, myocardial infarction; MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial Infarction.

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Supplemental Table 4. Baseline characteristics according to availability of MVO data (complete vs. missing). Data are mean ± SD, or n (%), unless otherwise stated.

	MVO data available (n=383)	MVO data missing (n=38)	P-value
Age	60.5 ± 10.0	61.9 ± 12.8	0.501
Male	327 (85%)	31 (82%)	0.482
White	359 (94%)	37 (97%)	0.715
Asian	22 (6%)	1 (3%)	0.710
Body mass index (kg/m^2)	28.1 ± 4.9	29.1 ± 4.9	0.235
Heart rate at presentation, beats/ min	72.4 ± 19.1	75.2 ± 18.6	0.396
Systolic blood pressure at presentation, mmHg	133.8 ± 25.1	135.4 ± 27.3	0.730
Diastolic blood pressure at presentation, mmHg	81.1 ± 14.7	80.0 ± 16.0	0.683
Anterior myocardial infarction	170 (44%)	15 (40%)	0.610
Hypertension	117 (31%)	18 (47%)	**0.044**
Hypercholesterolemia	83 (22%)	13 (34%)	0.103
Diabetes mellitus †	45 (12%)	8 (21%)	0.120
Smoking:			
Current	176 (46%)	21 (55%)	0.308
Former (stopped >3 months)	74 (19%)	7 (18%)	1.000
Never	133 (35%)	10 (26%)	0.370
Pre-existing maintenance medication:			
Aspirin	54 (14%)	9 (24%)	0.148
Statin	77 (20%)	14 (37%)	**0.023**
Beta blocker	33 (9%)	7 (18%)	0.074
ACE inhibitor or ARB	62 (16%)	11 (29%)	0.069
Symptom onset to arrival at primary PCI centre, median (IQR) hrs	2.2 (1.5, 3.2)	2.5 (1.7, 3.5)	0.354
Arrival at primary PCI centre to reperfusion, median (IQR) hrs	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	**0.002**

Initial blood results on admission:			
Hemoglobin, g/dL	145.6 ± 13.6	145.8 ± 10.3	0.892
Platelet count, $10^3/\mu L$	260.6 ± 60.9	270.9 ± 80.4	0.486
Creatinine, μmol/L	80.9 ± 17.7	78.6 ± 18.6	0.546
eGFR (ml/min/1.73m^2)	90.1 ± 20.4	92.5 ± 28.5	0.679

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Supplemental Table 5. Procedure characteristics according to availability of MVO data (complete vs. missing). Data are mean ± SD, or n (%), unless otherwise stated.

	MVO data available (n=383)	MVO data missing (n=38)	P-value
Culprit artery:			
Left anterior descending	176 (46%)	15 (40%)	0.497
Circumflex	44 (12%)	9 (24%)	**0.040**
Right coronary artery	163 (43%)	14 (37%)	0.606
>50% stenosis in ≥2 major coronary arteries	134 (35%)	10 (26%)	0.370
Initial TIMI coronary flow grade:			
≤1	337 (88%)	30 (79%)	0.126
≥2	46 (12%)	8 (21%)	0.126
Initial TIMI thrombus grade:			
3/4	76 (20%)	10 (26%)	0.398
5	307 (80%)	28 (74%)	0.398
American Heart Association culprit lesion type A	287 (75%)	30 (79%)	0.695
Culprit lesion plaque characterisation score ≥4	292 (76%)	29 (76%)	0.100
QCA lesion length pre-drug (mm)	26.8 ± 11.4	25.0 ± 10.0	0.305
Reperfusion achieved with balloon angioplasty	269 (70%)	32 (84%)	0.089
Balloon angioplasty pre-stent	354 (92%)	34 (90%)	0.523
Study drug delivered with thrombectomy catheter	278 (73%)	22 (58%)	0.062
Total number of stents deployed ≥2	115 (30%)	11 (29%)	1.000
Post-stent dilatation	337 (88%)	29 (76%)	0.072
Total length of stents deployed from QCA (mm)	34.0 ± 14.4	32.8 ± 14.9	0.638
QCA reference vessel diameter post-stent (mm)	3.2 ± 0.5	3.2 ± 0.4	0.535
Unfractionated heparin, median (IQR), U	10000.0 (75000.0, 13000.0)	8750.0 (7125.0, 12000.0)	0.135

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Activated clotting time (s)	281.7 ± 88.0	273.0 ± 94.0	0.673
Intravenous morphine	284 (74%)	27 (71%)	0.700
Inhaled oxygen (%)	55 (15%)	5 (15%)	1.000
Glycoprotein IIb/IIIa antagonist (%)	57 (15%)	9 (27%)	0.091
Duration of study drug infusion (min)	6.5 (2%)	6.4 (2%)	0.679

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Supplemental Table 6. Analysis of CMR parameters 2–7 days after primary PCI, by subgroups of TIMI flow grade (2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated.

	Treatment group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=134)*	Alteplase 10mg (n=131)*	Alteplase 20mg (n=136)*	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) Vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
MVO presence (n/ total) (a)								
TIMI 2 flow	13/36 (36.1)	22/37 (59.5)	21/43 (48.8)	2.58 (1.00, 6.65) p=0.051	1.66 (0.67, 4.13) p=0.257	0.153	2.06 (0.92, 4.62) p=0.081	**0.022**
TIMI 3 flow	41/85 (48.2)	29/80 (36.3)	33/83 (39.8)	0.61 (0.33, 1.14) p=0.119	0.72 (0.39, 1.34) p=0.298		0.66 (0.39, 1.13) p=0.128	
MVO extent (% of LV mass)† (b)								
TIMI 2 flow	3.0 ± 6.2	2.8 ± 3.9	5.2 ± 7.2	0.26 (-0.36, 0.89) p=0.405	0.55 (-0.18, 1.27) p=0.136	0.243	0.43 (-0.17, 1.02) p=0.158	0.107
TIMI 3 flow	2.2 ± 3.4	2.4 ± 4.8	2.3 ± 4.3	-0.09 (-0.49, 0.31) p=0.661	-0.06 (-0.45, 0.34) p=0.777		-0.07 (-0.42, 0.27) p=0.677	
Myocardial haemorrhage presence (n/ total) (a)								
TIMI 2 flow	10/33 (30.0)	20/35 (57.1)	21/43 (48.8)	3.05 (1.12, 8.31) **p=0.029**	2.14 (0.82, 5.62) p=0.121	0.054	2.55 (1.07, 6.06) **p=0.034**	**0.009**
TIMI 3 flow	39/82 (47.6)	28/76 (36.8)	30/81 (37.0)	0.64 (0.34, 1.21) p=0.168	0.66 (0.35, 1.23) p=0.188		0.65 (0.38, 1.11) p=0.117	
Myocardial haemorrhage extent (% LV mass)† (c)								
TIMI 2 flow	2.0 ± 5.7	2.0 ± 2.9	4.2 ± 6.0	1.95 (-0.33, 4.24) p=0.093	1.98 (-0.74, 4.69) p=0.151	0.132	0.50 (-0.04, 3.04) p=0.287	0.362
TIMI 3 flow	1.4 ± 2.8	1.8 ± 3.6	1.5 ± 3.8	0.29 (-1.00, 1.58) p=0.656	0.11 (-1.16, 1.38) p=0.867		0.20 (-0.91, 1.31) p=0.726	
Infarct size (% LV mass) (c)								
TIMI 2 flow	28.5 ± 16.4	30.5 ± 12.7	31.9 ± 13.5	2.46 (-3.91, 8.82) p=0.445	3.00 (-3.48, 9.48) p=0.359	0.158	2.69 (-2.67, 8.04) p=0.322	0.085
TIMI 3 flow	26.0 ± 12.6	24.7 ± 11.7	22.8 ± 12.3	-1.35 (-4.92, 2.22) p=0.460	-2.68 (-6.22, 0.86) p=0.138		-2.03 (-5.09, 1.03) p=0.195	
Myocardial salvage index (c)								
TIMI 2 flow	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	-0.05 (-0.17, 0.08) p=0.464	-0.07 (-0.18, 0.04) p=0.205	0.201	-0.06 (-0.15, 0.04) p=0.245	0.117
TIMI 3 flow	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.02 (-0.05, 0.09) p=0.593	0.04 (-0.03, 0.11) p=0.254		0.03 (-0.03, 0.09) p=0.329	

Supplemental Table 7. Analysis of MVO extent (% LV mass) 2-7 days after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration, with treatment effects derived by bootstrapping (10,000 replicates, stratified by the location of myocardial infarction).

	Treatment Effect on MVO extent		Treatment Effect on MVO extent
	Alteplase 10mg vs placebo	Alteplase 20mg vs. placebo	Alteplase (10mg or 20mg) vs. placebo
	Estimate (95% CI) p-value	Estimate (95% CI) p-value	Estimate (95% CI), p-value
TIMI flow ≤2	2.19 (-1.40, 4.08) p=0.284	3.37 (0.77, 6.89) **p=0.016**	2.80 (-0.09, 5.51) p=0.057
TIMI 3 flow	1.96 (-0.65, 3.20) p=0.237	1.91 (-0.74, 3.01) p=0.287	1.99 (-0.57, 2.92) p=0.246

Missing data: MVO extent (n=38).

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Supplemental Table 8. Analysis of electrocardiographic, biochemical and angiographic parameters, by subgroups of TIMI flow grade (≤2 vs. 3) at the time of study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated.

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)*	Alteplase 10 mg (n=136)*	Alteplase 20 mg (n=143)*	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
Absolute % ST-segment resolution 60 min (a)								
TIMI flow ≤2	45.0 ± 44.3	40.6 ± 52.1	37.7 ± 43.3	-4.43 (-21.96, 13.1) p=0.621	-7.37 (-24.21, 9.47) p=0.392	0.671	-6.02 (-20.91, 8.87) p=0.429	0.789
TIMI 3 flow	50.7± 36.4	44.4 ± 41.8	50.5 ± 46.0	-6.89 (-20.50, 6.73) p=0.322	-0.34 (-13.59, 12.91) p=0.960		-3.44 (-14.99, 8.11) p=0.560	
Troponin T (ng/mL) AUC, 0–24 hours (b)								
TIMI flow ≤2	2.66 [1.10, 5.20]	2.94 [1.73, 6.86]	4.60 [1.20, 8.19]	1.67 (0.96, 2.89) p=0.071	1.83 (1.07, 3.12) **p=0.029**	0.662	1.75 (1.09, 2.80) **p=0.021**	0.402
TIMI 3 flow	3.16 [1.16, 5.76]	2.67 [1.53, 5.71]	3.47 [1.57, 6.30]	1.38 (0.92, 2.08) p=0.120	1.34 (0.90, 2.00) p=0.151		1.36 (0.96, 1.92) p=0.082	
TIMI coronary flow grade post-PCI ≤2 (c)								
TIMI flow ≤2	19 (38.0)	15 (30.6)	22 (40.0)	0.72 (0.31, 1.66) p=0.432	1.09 (0.50, 2.39) p=0.838	0.134	0.90 (0.45, 1.81) p=0.762	0.071
TIMI 3 flow	5 (5.4)	12 (13.8)	11 (12.5)	2.80 (0.95, 8.34) p=0.064	2.53 (0.84, 7.61) p=0.099		2.66 (0.98, 7.27) p=0.056	
Corrected TIMI frame count post-PCI (b)								
TIMI flow ≤2	26.5 [17.4, 39.4]	22.4 [15.5, 35.9]	28.0 [21.8, 40.5]	0.91 (0.73, 1.13) p=0.372	1.07 (0.87, 1.32) p=0.534	0.095	0.99 (0.82, 1.19) p=0.902	0.276
TIMI 3 flow	17.7 [12.0, 24.0]	20.0 [14.0, 26.0]	17.4 [12.9, 24.0]	1.18 (1.00, 1.39) **p=0.049**	1.08 (0.92, 1.26) p=0.377		1.12 (0.98, 1.29) p=0.099	
Myocardial perfusion grade post-PCI ≤1 (c)								
TIMI flow ≤2	24 (48.0)	23 (46.9)	33 (60.0)	0.95 (0.42, 2.13) p=0.895	1.65 (0.75, 3.66) p=0.214	0.050	1.27 (0.63, 2.54) p=0.501	0.634
TIMI 3 flow	31 (33.7)	36 (41.4)	23 (26.1)	1.43 (0.77, 2.65) p=0.264	0.71 (0.37, 1.37) p=0.308		1.02 (0.59, 1.77) p=0.932	

(a) Treatment effect estimates reported as mean differences between groups.

(b) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

(c) Treatment effect estimates reported as odds ratios between groups, from a logistic regression model.

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The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.
*Missing data: % ST-segment resolution (n=43); Troponin AUC (n=115); corrected TIMI frame count post-PCI (n=2).

Abbreviations: AUC, area-under-the-curve; CI, confidence interval; IQR, interquartile range; MI, myocardial infarction; SD, standard deviation; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

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Supplemental Table 9. Analysis of coagulation variables, at 2 hours, at 24 hours, and at 24 hours compared to baseline, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are median [IQR], unless otherwise stated.

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)*	Alteplase 10mg (n=136)*	Alteplase 20mg (n=143)*	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
Fibrinogen (g/L) 2 hours post-PCI (a)								
TIMI flow ≤2	3.3 [2.7, 4.0]	3.2 [2.6, 3.6]	3.1 [2.7, 3.6]	0.98 (0.88, 1.1) p=0.784	0.94 (0.84, 1.05) p=0.250	0.684	0.96 (0.87, 1.05) p=0.391	0.955
TIMI 3 flow	3.3 [2.8, 3.9]	3.0 [2.6, 3.8]	3.3 [2.8, 3.7]	0.96 (0.88, 1.04) p=0.279	0.97 (0.89, 1.05) p=0.441		0.96 (0.90, 1.03) p=0.282	
Plasminogen (U/dL) 2 hours post-PCI (b)								
TIMI flow ≤2	95.0 [88.3, 101.0]	91.0 [81.5, 100.8]	83.5 [74.8, 92.0]	-2.66 (-8.56, 3.25) p=0.378	-10.88 (-16.52, -5.25) **p<0.001**	0.378	-7.16 (-12.23, -2.09) **p=0.006**	0.623
TIMI 3 flow	96.0 [87.0, 104.5]	88.0 [80.0, 98.0]	84.0 [77.0, 92.0]	-6.90 (-11.20, -2.66) **p=0.002**	10.49 (-14.73, -6.25) **p<0.001**		-8.74 (-12.45, -5.03) **p<0.001**	
Fibrin D-dimer (ng/mL) 2 hours post-PCI (a)								
TIMI flow ≤2	101.0 [69.5, 138.3]	319.5 [215.5, 633.0]	513.5 [266.8, 831.5]	3.64 (2.53, 5.22) **p<0.001**	4.91 (3.48, 6.93) **p<0.001**	0.563	4.29 (3.15, 5.83) p<0.001	0.299
TIMI 3 flow	117.0 [74.8, 169.0]	354.0 [224.0, 593.0]	421.0 [275.5, 641.5]	3.15 (2.43, 4.09) **p<0.001**	3.88 (3.00, 5.03) **p<0.001**		3.50 (2.80, 4.39) p<0.001	
Prothrombin fragment F_{1+2} (pmol/L) 2 hours post-PCI (a)								
TIMI flow ≤2	165.0 [134.0, 220.8]	161.1 [124.9, 260.8]	201.5 [147.4, 303.0]	1.20 (0.92, 1.57) p=0.183	1.22 (0.94, 1.57) p=0.136	0.909	1.21 (0.96, 1.52) p=0.103	0.925
TIMI 3 flow	155.5 [124.1, 267.0]	200.3 [144.0, 328.2]	199.1 [153.2, 303.0]	1.26 (1.04, 1.53) **p=0.019**	1.19 (0.98, 1.44) p=0.078		1.23 (1.04, 1.45) **p=0.017**	
Tissue plasminogen activator (ng/mL) 2 hours post-PCI (a)								
TIMI flow ≤2	11.0 [8.3, 13.0]	14.0 [11.0, 16.0]	15.0 [12.0, 19.3]	1.26 (1.00, 1.59) p=0.056	1.45 (1.16, 1.82) **p=0.001**	0.977	1.36 (1.11, 1.66) **p=0.003**	0.869
TIMI 3 flow	11.0 [9.0, 13.0]	13.0 [11.0, 17.0]	14.0 [12.0, 16.5]	1.30 (1.10, 1.54) **p=0.003**	1.48 (1.25, 1.76) **p<0.001**		1.39 (1.20, 1.61) **p<0.001**	
Fibrinogen (g/L) 24 hours post-PCI (a)								
TIMI flow ≤2	3.6 [3.0, 4.5]	3.6 [3.1, 4.4]	3.6 [3.0, 4.4]	1.03 (0.92, 1.16) p=0.576	0.99 (0.89, 1.11) p=0.927	0.384	1.01 (0.92, 1.12) p=0.791	0.176
TIMI 3 flow	3.8 [3.3, 4.6]	3.5 [2.8, 4.3]	3.5 [3.0, 4.1]	0.94 (0.86, 1.02) p=0.143	0.92 (0.85, 1.00) p=0.063		0.93 (0.87, 1.00) p=0.052	
Plasminogen (U/dL) 24 hours post-PCI (b)								
TIMI flow ≤2	91.0 [86.0, 102.0]	91.6 [84.8, 99.3]	86.0 [77.0, 94.0]	0.14 (0.00, 45.15) p=0.506	0.00 (0.00, 0.34) **p=0.021**	0.230	0.01 (0.00, 1.85) p=0.086	0.519
TIMI 3 flow	96.0 [83.0, 107.0]	88.0 [77.0, 99.3]	90.0 [80.0, 96.0]	0.00 (0.00, 0.07) **p=0.002**	0.00 (0.00, 0.16) **p=0.005**		0.00 (0.00, 0.06) **p<0.001**	

Fibrin D-dimer (ng/mL) 24 hours post-PCI (a)								
TIMI flow ≤2	103.0 [59.0, 150.0]	162.0 [112.0, 371.8]	224.0 [151.0, 344.0]	2.05 (1.46, 2.87) **p<0.001**	2.11 (1.51, 2.94) **p<0.001**	0.205	2.08 (1.55, 2.78) **p<0.001**	0.078
TIMI 3 flow	130.0 [80.5, 201.5]	190.0 [112.8, 379.0]	224.0 [133.0, 325.0]	1.44 (1.13, 1.85) **p=0.004**	1.56 (1.23, 1.99) **p<0.001**		1.50 (1.22, 1.85) **p<0.001**	
Prothrombin fragment F_{1+2} (pmol/L) 24 hours post-PCI (a)								
TIMI flow ≤2	197.0 [145.0, 262.2]	191.7 [129.7, 297.3]	204.0 [155.0, 321.0]	1.04 (0.80, 1.36) p=0.750	1.21 (0.93, 1.58) p=0.159	0.643	1.13 (0.89, 1.42) p=0.319	0.802
TIMI 3 flow	226.0 [153.6, 334.0]	226.8 [173.5, 324.6]	234.0 [166.4, 327.7]	1.09 (0.89, 1.32) p=0.404	1.08 (0.89, 1.31) p=0.416		1.08 (0.92, 1.28) p=0.336	
Tissue plasminogen activator (ng/mL) 24 hours post-PCI (a)								
TIMI flow ≤2	10.0 [8.0, 12.0]	11.0 [8.8 12.0]	10.0 [8.0, 13.0]	1.02 (0.84, 1.24) p=0.829	1.05 (0.87, 1.28) p=0.594	0.803	1.04 (0.88, 1.23) p=0.666	0.627
TIMI 3 flow	9.0 [7.0, 11.5]	10.0 [8.0 12.0]	10.0 [8.0, 12.0]	1.04 (0.91, 1.20) p=0.549	1.14 (0.99, 1.31) p=0.068		1.09 (0.97, 1.23) p=0.152	
Ratio of fibrinogen at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.12 [1.00, 1.26]	1.17 [1.10, 1.35]	1.16 [1.00, 1.37]	1.08 (0.99, 1.17) p=0.077	1.05 (0.96, 1.14) p=0.296	**0.040**	1.06 (0.99, 1.14) p=0.107	**0.013**
TIMI 3 flow	1.20 [1.00, 1.33]	1.10 [0.90, 1.35]	1.11 [1.00, 1.25]	0.95 (0.89, 1.01) p=0.101	0.95 (0.89, 1.00) p=0.071		0.95 (0.90, 1.00) **p=0.044**	
Change in plasminogen (U/dL) at 24 hours relative to baseline (b)								
TIMI flow ≤2	1.0 [-4.0, 5.5]	-3.0 [-9.0, 4.0]	-6.0 [-11.5, -2.3]	-2.57 (-6.46, 1.32) p=0.197	-7.05 (-10.89, -3.21) **p<0.001**	0.074	-4.87 (-8.25, -1.49) **p=0.005**	0.239
TIMI 3 flow	2.0 [-3.0, 6.0]	-6.5 [-10.3, 0.0]	-7.0 [-11.8, -0.3]	-7.52 (-10.36, -4.67) **p<0.001**	-7.22 (-10.04, -4.40) **p<0.001**		-7.37 (-9.81, -4.93) **p<0.001**	
Ratio of fibrin D-dimer at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.1 [0.8, 1.3]	1.8 [1.2, 3.3]	1.6 [1.0, 2.7]	2.01 (1.46, 2.77) **p<0.001**	1.67 (1.22, 2.30) **p=0.002**	**0.019**	1.83 (1.38, 2.42) **p<0.001**	0.249
TIMI 3 flow	1.3 [0.9, 1.7]	1.7 [1.0, 2.5]	2.2 [1.4, 3.4]	1.26 (1.00, 1.59) p=0.055	1.76 (1.40, 2.22) **p<0.001**		1.49 (1.22, 1.83) **p<0.001**	
Ratio of prothrombin fragment F_{1+2} at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.2 [1.0, 1.6]	1.5 [13.9, 2.0]	1.3 [1.0, 1.9]	1.26 0.93, 1.71) p=0.134	1.23 (0.91, 1.66) p=0.173	0.520	1.25 (0.96, 1.62) p=0.103	0.200
TIMI 3 flow	1.4 [0.9, 1.9]	1.4 [1.0, 1.6]	1.4 [1.2, 2.1]	0.87 (0.70, 1.09) p=0.219	1.16 (0.93, 1.45) p=0.182		1.01 (0.83, 1.22) p=0.937	
Ratio of tissue plasminogen activator at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.1 [0.9, 1.3]	1.2 [1.0, 1.3]	1.1 [0.9, 1.3]	1.05 (0.78, 1.41) p=0.761	0.98 (0.73, 1.32) p=0.894	0.454	1.01 (0.78, 1.31) p=0.926	0.444
TIMI 3 flow	0.9 [0.8, 1.2]	1.0 [0.8, 1.2]	1.1 [0.8, 1.4]	0.84 (0.68, 1.05) p=0.121	0.95 (0.76, 1.18) p=0.635		0.89 (0.74, 1.08) p=0.240	

(a) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

(b) Treatment effect estimates reported as mean differences between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

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*Missing data: coagulation parameters 2 hours post-PCI (n=75); coagulation parameters 24 hours post-PCI (n=71); change in coagulation parameters at 24 hours relative to baseline (n=97).

Abbreviations: IQR, inter quartile range; MI, myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

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