Sponsor:	**Name of Finished Product:**	**Name of Active Ingredient:**
Thrombolytic Science, LLC	HisproUK	mutant pro-urokinase

Title of Study:

SALVAGE-AMI: An open label, randomised study to evaluate the safety and preliminary efficacy of sequential 'physiological' fibrinolysis with low dose alteplase and mutant pro urokinase to establish early epicardial and microvessel patency in patients presenting with a ST-elevation myocardial infarction with an expected delay of at least one hour before undergoing mechanical reperfusion at a PCI capable hospital

Investigators/Study Centers:

This study was conducted in a high volume primary PCI-capable hospital linked to 2 non-PCI capable hospitals in South Wales, UK and was approved by the Medicines & Healthcare products Regulatory Agency (MHRA) as well as the Wales Ethics Committee in December 2023.

- Dr Vasim Farooq (CI and PI), University Hospital Wales, Cardiff
- Dr Mateusz Szmidt (PI), Prince Charles Hospital, Cardiff
- Dr Mayukhmoy Maiti (PI), Royal Glamorgan Hospital, Cardiff

Publication (reference):	**Phase of Development:**
Not applicable	Phase 2

Studied Period (years):

Date of first enrollment: 10-Oct-2024

Date of last completed: 07-Mar-2025 / Database lock: 30-Apr-2025

The recruitment period as stated in the clinical trial protocol was targeted to have completed within 10-12 months, anticipating 4-5 participants enrolled per month across 2 sites. However, the study was discontinued in March 2025 due to insufficient enrollment and protocol deviations that limited the evaluable dataset.

Objectives:

Primary Objective

Safety and preliminary efficacy of sequential 'physiological' fibrinolysis (low dose bolus alteplase followed by an infusion of mutant pro-urokinase (mproUK; previously referred to as HisproUK) in patients presenting with a ST-elevation myocardial infarction (STEMI), with an anticipated delay of at least one hour before being treated at a hospital capable of conducting percutaneous coronary intervention (PCI), i.e., PCI-capable hospital, for emergency mechanical reperfusion with primary PCI. Safety was assessed by changes in serum fibrinogen levels from baseline to 6 hours after randomisation.

Secondary Objectives

Efficacy and other safety outcomes were to be assessed by:

- Acute response to the IMP, namely reperfusion, thrombus, epicardial patency (coronary angiography) pre- and post- PCI, and microvascular patency (coronary physiology: index of microcirculatory resistance [IMR]) at end of index primary PCI procedure
- Percentage ST segment resolution on ECG taken at baseline, immediately prior to primary PCI and 1 hour post primary PCI
- Acute infarct characteristics on contrast-enhanced cardiac MRI at Day 2-4
- Final infarct size on contrast-enhanced cardiac MRI at Day 30 (range 23-44 days)
- Selvester score of 30-day infarct size on ECG taken at Day 30
- Mortality, readmission for heart failure and acute bleed rates at Day 30
- Assess the safety of the infused HisproUK
- Fibrinogen levels stratified by dose and infusion time of the HisproUK
- Measures of changes in fibrinogen (a measure of systemic fibrinolysis – depletion will increase the risk of bleeding) and fibrin D-dimer (fibrin degradation product) over a 24 hr. period following randomisation.

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- Patient-reported quality of life, angina status and disease outcome at Day 30 by the use of the HeartQOL survey as a disease specific QOL measure, the Seattle Angina Questionnaire (SAQ) and the Medical Outcome Study 36-item Short-Form Health Survey (MOS SF-36) as a generic measure.

Methodology:

Following verbal assent, patients were randomised (2:1) to either of: –

- the intervention arm of immediate sequential fibrinolysis, or

- the current standard of care

For all patients, transportation to the PCI-capable hospital was organized immediately on presentation with a STEMI. All patients received pretreatment with a loading dose of aspirin 300 mg. In the control arm pre-treatment with a loading dose of contemporary P2Y12 inhibitor (180 mg ticagrelor) was permitted as per the current standard of care in South Wales.

For all patients, a baseline fibrinogen and D-Dimer (fibrin degradation product) level, along with other safety bloods, were taken immediately upon presentation and shipped to the PCI-capable hospital for analysis.

For patients randomised to the intervention arm (sequential fibrinolysis treatment), a single dose of IMP (alteplase + HisproUK) was administered. The infusion time of HisproUK was dependent on the transfer time to the cath lab at the PCI-capable hospital.

Upon arrival at the PCI-capable hospital the patient was transferred to the cath lab table to undergo the primary PCI procedure. Immediately prior to the primary PCI procedure, a further serum fibrinogen and D-Dimer level were to be taken and analysed at the PCI-capable hospital.

For patients in the intervention arm, following administration of sequential fibrinolysis, the patient was to undergo primary PCI with administration of periprocedural unfractionated heparin (UFH). At the end of the primary PCI procedure the patient was administered a single 2.5 mg intravenous dose of fondaparinux.

Further serum fibrinogen and D-Dimer level samples were taken immediately after the primary PCI procedure, and at 6 hours and 24 hours after randomisation. For all patients, following the primary PCI procedure, a maintenance dose of aspirin 75 mg was to be continued indefinitely, and ticagrelor 90 mg bd for one year as per the standard of care for STEMI. Written informed consent was obtained as soon as possible from the patient or next of kin/legal guardian following the primary PCI procedure.

Number of Patients (planned and analysed):

This study was intended to recruit 48 patients in a 2:1 randomisation schedule, stratified by site, to achieve 32 patients treated with the IMP (HisproUK preceded by low dose alteplase) and 16 with standard of care.

The final enrollment included 4 patients treated with alteplase + HisproUK, 1 with standard care, and 1 who withdrew from the study prior to receiving study drug.

Diagnosis and Main Criteria for Inclusion:

Key entry criteria included presentation to an investigational site with an acute STEMI (symptom onset 0-6 hours) requiring mechanical reperfusion with primary PCI to one or more lesions; anticipated delay to primary PCI of at least one hour from presentation to treatment with emergency mechanical reperfusion at the PCI-capable hospital; no left bundle branch block or ventricular pacing, no known high risk of bleeding and not currently taking a P2Y12 inhibitor or oral anticoagulant therapy.

Test Product, Dose and Mode of Administration, Batch/Lot Number

For patients randomised to the intervention arm (sequential fibrinolysis treatment), a mini-bolus of low dose intravenous alteplase (5 mg) was to be followed by a continuous intravenous infusion of mutant pro-urokinase (HisproUK) at 40 mg/hr for 60-90 mins (40-60 mg in total). Upon arrival at the PCI-capable hospital the patient was transferred to the cath lab table to undergo the primary PCI procedure.

HisproUK Lot Number: 16-1845

Commercially available preparations of alteplase (10 mg vials) were used for the 5 mg mini-bolus.

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Duration of Treatment:

A single dose of IMP (alteplase + HisproUK) was administered to patients in the intervention arm.

The treatment and follow-up period for each study patient was 1 month, which included presentation with STEMI, PCI, cardiac MRI and 30 day follow-up.

Reference Therapy:

The comparator is the standard of care, i.e., urgent inter-hospital transfer to a PCI-capable hospital for primary PCI with or without standard dose full dose fibrinolysis. Where the anticipated delay, in the Investigators opinion, was expected to be >2 hours, full dose fibrinolysis was to be administered as per the European Society of Cardiology (ESC) guidelines.

All patients received pretreatment with a loading dose of aspirin 300 mg.

Criteria for Evaluation of Efficacy and Safety

Primary Endpoint

Change from baseline serum fibrinogen levels to 6 hours after randomisation.

Secondary Endpoints

- Acute response to the IMPs:
  - Coronary angiography measures of reperfusion and thrombus (pre and post- primary PCI procedure).
    - TIMI flow, TIMI myocardial blush grade, TIMI frame count, TIMI thrombus grade
  - Epicardial patency (reference vessel diameter, minimum lumen diameter, and percentage diameter stenosis) from coronary angiography pre- (immediately after coronary angiogram) and post-PCI procedure.
  - Microvascular patency (coronary physiology – index-of microcirculatory resistance [IMR]) at end of index primary PCI procedure.
- Percentage ST segment resolution on ECG taken at baseline, immediately prior to the primary PCI procedure, and 1 hour post primary PCI.
- Comparison of acute infarct characteristics on contrast-enhanced cardiac MRI at day 2-4
  - Myocardial salvage index (MSI) as a surrogate measure of therapeutic benefit
  - Late gadolinium enhancement (LGE, % LV mass) volume (acute infarct size)
  - Incidence and extent of microvascular obstruction (MVO) and/or haemorrhage, expressed as a percentage of left ventricular mass
  - Left ventricular end–diastolic volume, left ventricular end–systolic volume, and left ventricular ejection fraction
- Assessment of the final infarct size on contrast-enhanced cardiac magnetic resonance imaging (MRI) at Day 30 (range 23-44 days).
- Selvester score of 30-day infarct size on the ECG taken at day 30.
- 30-day mortality, readmission for heart failure, and acute bleeds (BARC ≥3).
- Outcomes stratified by transfer times and dose of the IMPs administered.
- Changes in fibrinogen and D-Dimers levels from baseline, immediately pre- and post- primary PCI, 6 hrs* and 24 hrs post randomisation.
- Patient reported outcomes (PROM) for Heart Related Quality of Life (HRQoL), Medical Outcome Study 36 item health Survey (MOS SF-36) and the Seattle Angina Questionnaires (SAQ) at day 30.

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Safety Endpoints

- Serious Adverse Events and follow-ups including:
  - Any pre-, peri- or post-procedural (up to 30 days) major bleeding (Bleeding Academic Research Consortium [BARC] ≥3)
  - Recurrent infarct(s)
  - Heart failure
  - Death
- Adverse events including, but not limited to:
  - Vascular access site complications
  - Coagulation results

Statistical Methods

Statistical methods were specified in the protocol. However, the study was discontinued due to poor enrollment and protocol deviations, resulting in a limited evaluable dataset. No statistical analyses were conducted.

Summary – Conclusions

Disposition, Demographics and Other Pertinent Baseline Characteristics

The final enrollment included 4 patients treated with alteplase + HisproUK, 1 with standard of care, and 1 patient who was randomized to receive alteplase + HisproUK but withdrew before receiving study drug. Patient disposition and baseline characteristics are provided in Table 1, below.

Efficacy Results

The Sponsor discontinued the trial in March 2025 due to insufficient patient accrual and protocol deviations that greatly reduced the available data for the primary outcome analyses. Efficacy analyses were not performed.

Safety Results

No significant safety concerns were reported or identified by the Sponsor throughout the duration of the trial. All 6 patients completed participation per protocol and were instructed to contact their primary physicians for post-research care if required. Two patients each experienced one AE, and a third patient experienced 4 AEs (Table 2). Five of the AEs were mild and one was moderate; none were considered related to the Study Drug. No SAEs occurred.

Conclusions

This final clinical study report (CSR) is in synoptic format as the SALVAGE_AMI trial is considered an incomplete study, having enrolled fewer than one-third of intended participants.

The study was discontinued due to poor enrollment and protocol deviations, resulting in a limited evaluable dataset. No significant safety concerns were identified during the study. No statistical review was performed on the study data as no statistical analysis were conducted. Furthermore, the limited number of treated patients does not permit conclusions beyond the safety observations reported in Table 2, below.

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Table 1. Phase 2 SALVAGE-AMI Patient Demographics and Disposition

Patient ID	Randomised Treatment Group	Patient Status	Sex	Age	Race
826002006	Intervention (sequential fibrinolysis)	Completed	Male	62	Caucasian
826002005	Intervention (sequential fibrinolysis)	Withdrawn (Patient withdrew consent before dosing)	-	-	Caucasian
826003003	Intervention (sequential fibrinolysis)	Completed	Male	56	Caucasian
826002004	Intervention (sequential fibrinolysis)	Completed	Male	37	Caucasian
826002001	Intervention (sequential fibrinolysis)	Withdrawn (Patient withdrew consent after Day 2-4 Visit and did not attend Day 30 Visit)	Male	58	Caucasian
826003001	Control (Standard of Care)	Completed	Male	50	Caucasian

Table 2. Phase 2 SALVAGE-AMI Adverse Event Data

Patient ID	Randomised Treatment Group	AE Description	AE Intensity	Serious AE?	Relationship to Study Drug	AE Outcome
826002001	Intervention (sequential fibrinolysis)	Viral illness	Mild	No	Not Related	Ongoing
826002004	Intervention (sequential fibrinolysis)	Cold feeling all over body	Mild	No	Not Related	Recovered with Sequelae
826002006	Intervention (sequential fibrinolysis)	Pyrexia	Mild	No	Not Related	Recovered with No Sequelae
		Chest Pain	Mild	No	Not Related	Recovered with No Sequelae
		Chest pain	Mild	No	Not Related	Recovered with No Sequelae
		Reduced Left ventricle Ejection Fraction	Moderate	No	Not Related	Recovered with No Sequelae

AE = adverse event

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