PCR e-Course 2021 LATE BREAKING SCIENCE

Pharmacokinetics, pharmacodynamics, and tolerability of subcutaneous administration of a novel glycoprotein IIb/IIIa inhibitor, RUC-4, in patients with ST-segment elevation myocardial infarction

Willem L. Bor[1], MD; Kai L. Zheng[1], MD; Anne H. Tavenier[2], MD; C. Michael Gibson[3], MD; Christopher B. Granger[4], MD; Ohad Bentur[5], MD; Rita Lobatto[6], MD, MPH; Sonja Postma[7], PhD; Barry S. Coller[5], MD; Arnoud W.J. van 't Hof[2,8,9,10], MD, PhD; Jurrien M. ten Berg[1,8,9]*, MD, PhD

1. Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands; 2. Isala Hospital, Zwolle, the Netherlands; 3. Beth Israel Deaconess Medical Center, Boston, MA, USA; 4. Duke University School of Medicine, Durham, NC, USA; 5. Allen and Frances Adler Laboratory of Blood and Vascular Biology, Rockefeller University, New York, NY, USA; 6. RP & L Consultancy B.V., Wassenaar, the Netherlands; 7. Diagram B.V., Zwolle, the Netherlands; 8. University Medical Center Maastricht, Maastricht, the Netherlands; 9. Cardiovascular Research Institute Maastricht (CARIM), Maastricht, the Netherlands; 10. Zuyderland Hospital, Heerlen, the Netherlands

Willem L. Bor and Kai L. Zheng contributed equally to this work.

This paper also includes supplementary data published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-21-00287

KEYWORDS

- adjunctive pharmacotherapy
- clinical research
- clinical trials
- innovation
- STEMI

Abstract

Background: Pre-hospital platelet inhibition in patients with ST-segment elevation myocardial infarction (STEMI) may improve outcomes. RUC-4 is a novel, second-generation glycoprotein IIb/IIIa inhibitor designed for first-point-of-medical-contact treatment for STEMI by subcutaneous injection.

Aims: The open-label, phase 2A, CEL-02 trial aimed to assess the pharmacodynamics (PD), pharmacokinetics (PK), and tolerability of RUC-4 in STEMI patients undergoing primary PCI (pPCI).

Methods: A total of 27 STEMI patients received a weight-adjusted subcutaneous injection of RUC-4 before pPCI in escalating doses (0.075 mg/kg [n=8], 0.090 mg/kg [n=9], or 0.110 mg/kg [n=10]).

Results: The primary PD endpoint of high-grade (≥77%) inhibition of the VerifyNow iso-TRAP assay at 15 minutes was met in 3/8, 7/8, and 7/8 patients in the three cohorts with a dose-response relationship (mean inhibition [min - max] of 77.5% [65.7%-90.6%], 87.5% [73.8%-93.1%], and 91.7% [76.4%-99.3%], respectively; p_{trend}=0.002). Fifty percent (50%) inhibition remained after 89.1 (38.0-129.7), 104.2 (17.6-190.8), and 112.4 (19.7-205.0) minutes. Injection site reactions or bruising were observed in 1 (4%) and 11 (41%) patients, respectively. Mild access-site haematomas occurred in 6 (22%), and severe access-site haematomas occurred in 2 patients (7%). No thrombocytopaenia was observed within 72 hours post dose.

Conclusions: In patients with STEMI, a single subcutaneous dose of RUC-4 at 0.075, 0.090, and 0.110 mg/kg showed dose-response high-grade inhibition of platelet function within 15 minutes.

**Corresponding author: Department of Cardiology, St Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, the Netherlands. E-mail: jurtenberg@gmail.com*

SUBMITTED ON 30/03/2021 - REVISION RECEIVED ON 1st 23/04/2021 - 2nd 30/04/2021 - ACCEPTED ON 07/05/2021

Abbreviations

ACS	acute coronary syndrome
ACT	activated clotting time
ADP	adenosine diphosphate
AE	adverse event
BARC	Bleeding Academic Research Consortium
CABG	coronary artery bypass grafting
CAD	coronary artery disease
CCL	cardiac catheterisation laboratory
CI	confidence interval
DAPT	dual antiplatelet therapy
DOAC	direct oral anticoagulant
GPI	glycoprotein IIb/IIIa inhibitor(s)
IV	intravenous
LAD	left anterior descending artery
LCx	circumflex branch of left coronary artery
LTA	light transmission aggregometry
MI	myocardial infarction
OHCA	out-of-hospital cardiac arrest
pPCI	primary percutaneous coronary intervention
RCA	right coronary artery
SAE	serious adverse event
STEMI	ST-segment elevation myocardial infarction
TIMI	Thrombolysis In Myocardial Infarction
TRAP	thrombin receptor activating peptide
VKA	vitamin K antagonist

Introduction

Platelet activation and thrombus formation play a pivotal role in the pathophysiology of acute coronary syndromes (ACS). Current guidelines recommend treatment of ACS patients with dual antiplatelet therapy (DAPT, aspirin and a $P2Y_{12}$ inhibitor) to reduce thrombotic events[1]. However, the onset of action of oral $P2Y_{12}$ inhibitors is slow, often requiring as long as 4-6 hours in the setting of ST-segment elevation myocardial infarction (STEMI)[2], independent of higher loading doses[3]. Late onset of action of oral $P2Y_{12}$ inhibition may be due to delayed gastrointestinal absorption, cardiogenic shock, vomiting of the loading dose, or concomitant use of opioids[4,5]. Despite the faster platelet inhibition by either chewed or crushed oral $P2Y_{12}$ inhibitors, an initial gap in the optimal platelet inhibition still exists, especially in the crucial first hours of STEMI[6-9]. Early, parenteral platelet inhibition with glycoprotein IIb/IIIa inhibitors (GPI) in STEMI patients has been shown to improve pre-primary percutaneous coronary intervention (pPCI) target vessel patency, post-pPCI ST-segment resolution, and patient outcomes[10-13]. However, conventional GPI are associated with increased bleeding and thrombocytopaenia[14,15]. They also require continuous intravenous (IV) infusion, which is inconvenient in the acute, pre-hospital phase. Hence, there is an unmet need in the acute phase of STEMI for easily administered platelet inhibitors that on the one hand are rapidly acting and potent, thereby reaching maximal efficacy quickly, and on the other hand have limited duration of action, thereby reducing the risk of bleeding.

RUC-4 is a novel, small-molecule, second-generation GPI that was designed for first-point-of-medical-contact treatment for STEMI – administered subcutaneously by a single injection[16,17] and potentially by auto-injector. In Phase 1 studies in healthy subjects and patients with stable coronary artery disease (CAD) treated with aspirin, RUC-4 was well tolerated up to 0.075 mg/kg and achieved high-grade inhibition of platelet function measured by light transmission aggregometry (LTA) in response to 20 µM adenosine diphosphate (ADP) within 15 minutes, with return of platelet function within two hours[18,19]. Previous studies of GPI found that 80% inhibition of LTA stimulated with 20 µM ADP correlated with *in vivo* antithrombotic effects in patients undergoing PCI and improved outcomes[20].

As the next step in the development of RUC-4, we conducted an open-label, dose escalating, Phase 2A study (CEL-02) to assess the pharmacodynamic (PD) and pharmacokinetic (PK) properties of a weight-adjusted dose of RUC-4 that was administered to STEMI patients in the cardiac catheterisation laboratory (CCL). Furthermore, the tolerability of RUC-4 will be described in this population.

Editorial, see page 362

Methods

This was a prospective, open-label, single-centre, Phase 2A study of a single subcutaneous dose of RUC-4 in patients with STEMI undergoing pPCI. The study was conducted at the St. Antonius Hospital, Nieuwegein, the Netherlands, approved by the local independent ethics committee, and conducted in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice, and local laws and regulations. The study is registered on ClinicalTrials.gov (NCT04284995). The full study protocol is attached in **Supplementary Appendix 1**. All patients provided verbal consent on arrival at the CCL prior to any study procedures; written informed consent was obtained before hospital discharge.

Based on the PK/PD data from the Phase 1 study, patients were enrolled in three cohorts of eight patients, and received a weight-adjusted dose of RUC-4, starting at 0.075 mg/kg, with dose escalation to 0.090 mg/kg and 0.110 mg/kg in the second and third cohort, respectively[18]. Dose escalation was based on a review by the Safety Review Committee after assessing the results from eight evaluable patients, each with at least 15 days of follow-up.

STUDY PATIENTS

Eligible patients were adults presenting for pPCI with STEMI within six hours of symptom onset. Patients were not eligible if they met one of the following criteria: high suspicion of a stent thrombosis causing the current STEMI; high suspicion of type 2 myocardial infarction (MI); out-of-hospital cardiac arrest; cardiogenic shock; persistent severe hypertension; severe renal dysfunction; clinically important anaemia; coagulation abnormality; bleeding disorder; prior haemorrhagic or ischaemic stroke; estimated body weight <52 kg or >120 kg; or use of concomitant IV GPI or oral anticoagulants. The complete inclusion and exclusion criteria are listed in the study protocol **(Supplementary Appendix 1)**.

STUDY PROCEDURES

All patients received standard of care treatment for STEMI in the ambulance, including aspirin (500 mg IV), ticagrelor (180 mg oral), and unfractionated heparin (UFH; 5,000 IU IV). Upon arrival at the CCL, after consent, a pre-dose blood sample was obtained, and patients were administered a single subcutaneous dose of RUC-4 in the outer aspect of the upper arm. If UFH was administered pre-hospital, an activated clotting time (ACT) was measured and, if the ACT was <200 seconds, additional UFH was recommended. For PD/PK assessments, additional blood samples were obtained at 15, 30, 45, 60, 90, 120 and 180 minutes post dose; a 240-minute PD time point was added for the second and third cohorts. Platelet counts were assessed up to 72 hours post dose.

PHARMACODYNAMIC ASSESSMENTS

Samples were collected in vacuum tubes (Greiner Bio-One 2 mL, 3.2% sodium citrate) for platelet function measurements. The primary PD endpoint of inhibition of platelet function was measured with the VerifyNow $P2Y_{12}$ assay employing the thrombin receptor activating peptide (iso-TRAP) channel[21] with the objective of achieving 77% or greater inhibition of platelet function at 15 minutes. In studies submitted for publication (Bentur et al), we found that 77% inhibition of the iso-TRAP channel corresponds to 80% inhibition of LTA stimulated by 20 µM ADP, the value that has been most closely correlated with antithrombotic effects *in vivo* using other GPI[20] **(Supplementary Appendix 2)**. We also determined that the iso-TRAP assay was not affected by aspirin, and only inhibited by about 20% at peak levels of ticagrelor achieved *in vivo*. We also assessed the results from another channel in the $P2Y_{12}$ cartridge, termed the Base channel, which contains a higher concentration of iso-TRAP than the iso-TRAP channel (20 µM vs 3-4 µM) in addition to an activator of an additional thrombin receptor (PAR-4). We found that this assay is not affected by either aspirin or ticagrelor, even at peak levels of ticagrelor achieved *in vivo*, and that 77% inhibition of platelet function using this assay is also the equivalent of 80% inhibition of 20 µM ADP-induced LTA. The time to return to 50% platelet function was reported for the iso-TRAP and Base assays as a measure of the offset of RUC-4's antiplatelet effects.

PHARMACOKINETIC ASSESSMENTS

Samples for PK analysis were collected by adding 1 mL of whole blood to tubes containing 4 mL of pre-chilled 30% acetonitrile. After vortexing, samples were frozen at –80°C within two hours of blood collection. Samples from each cohort were analysed in batches as previously described[18]. PK parameters for RUC-4 and its most abundant metabolite, RUC-4-des-glycine, were calculated. For the non-compartmental PK analysis, all blood concentration levels below the level of quantification occurring prior to the first measurable concentration were set to zero. Values below the level of quantification occurring after the first measurable blood concentration were noted as missing. PK data reported include the whole blood RUC-4 concentration (ng/mL), the maximal RUC-4 concentration (C_{max}), and time to reach maximum RUC-4 concentration (t_{max}).

TOLERABILITY

Platelet counts were measured pre-dose and at 1, 24, and 72 hours post dose. Bleeding events were classified according to the Bleeding Academic Research Consortium (BARC) criteria[22]; intraprocedural thrombosis and injection site reactions were assessed up to 30 days post dose. All adverse events were classified Grade 1 to 5 as per Common Terminology Criteria for Adverse Events version 5.0.

STATISTICAL ANALYSIS

All statistical summaries are descriptive in nature. No hypothesis testing was performed for primary and secondary analyses.

For continuous data, summary statistics including number of observations (n), arithmetic mean and standard deviation (SD), median, quartile 1 (Q1), quartile 3 (Q3), minimum (min), and maximum (max) are presented. For categorical data, frequency counts and percentages are presented.

Descriptive statistics are presented for the PD/PK data at different time points and depicted graphically. A p_{trend} was calculated for percent inhibition at 15 minutes. An inverse estimation was performed for return to 50% platelet inhibition, C_{max} and t_{max} based on a mixed model with a continuous time effect and random intercept. Statistical analyses were performed using SAS, release 9.4 (SAS Institute, Cary, NC, USA).

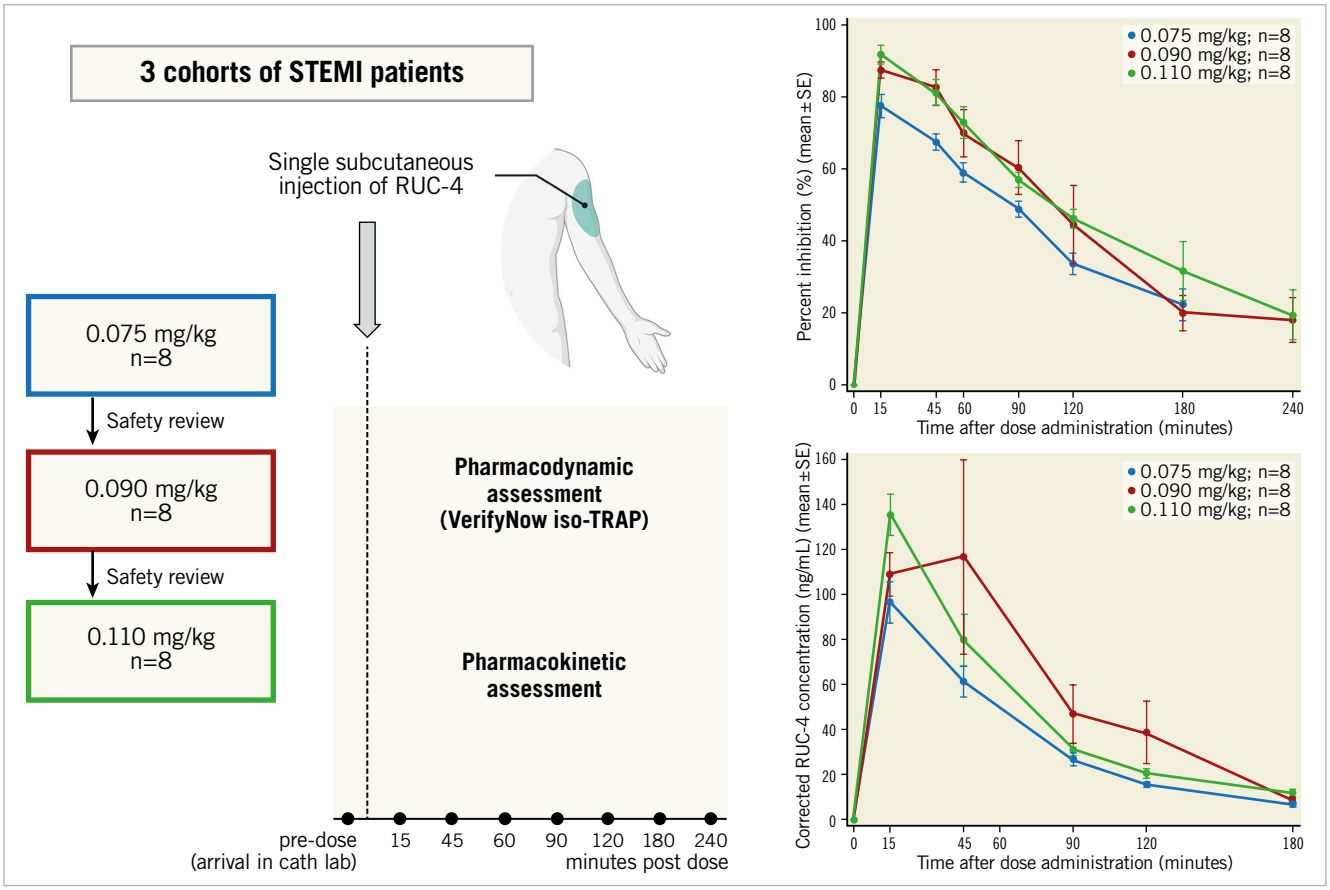
Results

BASELINE CHARACTERISTICS

Between June 2020 and October 2020, 27 patients were enrolled and received RUC-4 (0.075 mg/kg [n=8], 0.090 mg/kg [n=9] or 0.110 mg/kg [n=10]), upon arrival at the catheterisation laboratory, immediately before pPCI. Of these, 24 patients were evaluable for PD analysis and 25 were evaluable for PK analysis **(Central illustration)**. The reasons for being unevaluable were: withdrawal of informed consent (one patient, excluded for PK and PD), failed baseline sampling (one patient, excluded for PD), and use of wrong VerifyNow cartridges (one patient, excluded for PD).

Patient demographics, baseline characteristics, and concomitant medications are summarised in **Table 1**. The majority of patients were male (74%) and Caucasian (96%); their mean age was 62 (range 38-91) years. All patients presented with symptoms and ECG findings consistent with STEMI; however, in one patient ST-segment elevation was caused by perimyocarditis without CAD, and one patient had a spontaneous coronary artery dissection with spasm resulting in <50% stenosis. Thus, 25/27 (93%) patients were treated with pPCI for an acute coronary occlusion.

Almost all patients were concomitantly treated with aspirin (93%), ticagrelor (93%), and UFH (96%) in the ambulance. The ACT prior to pPCI was <200 seconds in 92% of the patients, and



Central illustration. *Single-dose subcutaneous RUC-4 induces a fast, potent, dose-dependent platelet inhibition in STEMI patients presenting for primary PCI. PCI: percutaneous coronary intervention; STEMI: ST-segment elevation myocardial infarction; TRAP: thrombin receptor activating peptide*

thus they received additional UFH during cardiac catheterisation. Flow was completely restored (Thrombolysis In Myocardial Infarction [TIMI] 3 flow) in 96% of patients who underwent PCI. One patient had a suboptimal result of PCI and a high thrombus load, and so received tirofiban bail-out treatment ~90 minutes later, at a time when the platelet inhibitory effect of RUC-4 was expected to be less than 50%.

PHARMACODYNAMICS

Figure 1 shows the platelet function findings 15 minutes after receiving RUC-4 for each patient in each cohort using both the iso-TRAP and Base channels. RUC-4 produced greater than 77% inhibition of platelet function in 3/8, 7/8, and 7/8 patients in the subsequent cohorts with the iso-TRAP assay, and 7/8, 8/8 and 8/8 patients reached greater than 77% inhibition of platelet function with the Base channel assay, respectively. The one patient in the third cohort who did not have 77% or more inhibition of platelet function with the iso-TRAP assay at 15 minutes had 76% inhibition of platelet function. The inhibition of platelet function was greatest at 15 minutes **(Figure 2, Figure 3)** and then began to decrease, reaching 50% inhibition on the iso-TRAP assay at 89.1 (95% confidence interval [CI]: 38.0-129.7), 104.2 (95% CI: 17.6-190.8) and 112.4 minutes (95% CI: 19.7-205.0) after administration in the cohorts, respectively. The comparable times with Base channel assay were 89.1 (95% CI: 36.9-141.3), 120.8 (95% CI: 6.2-235.4) and 128.9 minutes (95% CI: 41.6-216.2).

Figure 2 and **Figure 3** provide the aggregate inhibition of platelet function data with the iso-TRAP and Base channel assays, respectively, at all of the indicated time points. For the iso-TRAP assay, the mean (min-max) results at 15 minutes were 77.5% (65.7%-90.6%), 87.5% (73.8%-93.1%), and 91.7% (76.4%-99.3%), respectively (p_{trend}=0.002). The Base channel inhibition of platelet function at 15 minutes post dose with the three cohorts was 86.0% (74.2%-97.7%), 92.3% (86.8%-97.1%), and 93.0% (81.9%-98.6%), respectively (p_{trend}=0.029).

PHARMACOKINETICS

Following the RUC-4 injection a mean maximum blood concentration of (mean±SD) 96.7±26.0 ng/mL, 152.4±114.0 ng/mL, and 129.8±23.3 ng/mL was observed for the 0.075 mg/kg, 0.090 mg/kg, and 0.110 mg/kg cohorts, respectively **(Figure 2, Figure 3)**. These maximum whole blood concentrations were reached at mean 20 minutes post dose (t_{max}, median [min-max]: 15 [15-16] minutes, 15 [14-45] minutes, and 15 [13-45] minutes, respectively).

Table 1. Baseline characteristics.

Demographics		
Age, years		62 (13)
Female sex		7/27 (26%)
Caucasian race		26/27 (96%)
Body mass index, kg/m^2		26.3 (3.8)
Medical characteristics		
Diabetes mellitus		0/27 (0%)
Hypertension		10/27 (37%)
Hypercholesterolaemia		9/27 (33%)
Peripheral vascular disease		0/27 (0%)
Prior myocardial infarction		4/27 (15%)
Prior PCI		4/27 (15%)
Prior CABG		0/27 (0%)
History of stroke		0/27 (0%)
Haemoglobin, g/dL		14.0 (1.2)
Platelet count, 10^9/L		269 (66)
Creatinine clearance, mL/min/1.73 m^2		88 [73-90]
Clinical presentation		
STEMI		27/27 (100%)
Time from symptoms to RUC-4 administration, minutes		139 [88-143]
Time from RUC-4 administration to angiography, minutes		4 [3-5]
Ambulance pre-treatment		
Aspirin pre-treatment		25/27 (93%)
Time to RUC-4 administration, minutes		47 [54-39]
Ticagrelor pre-treatment		25/27 (93%)
Time to RUC-4 administration, minutes		47 [54-37]
Heparin pre-treatment (5,000 IU)		26/27 (96%)
Time to RUC-4 administration, minutes		47 [54-37]
Procedural characteristics		
Access site	Femoral	2/27 (7%)
	Radial	25/27 (93%)
Culprit vessel	LAD	12/27 (44%)
	LCx	4/27 (15%)
	RCA	10/27 (37%)
	none	1/27 (4%)
PCI performed		25/27 (93%)
Additional heparin during PCI		23/25 (92%)
Bail-out GPI (tirofiban)		1/25 (4%)
TIMI flow pre	0	11/25 (44%)
	1	1/25 (4%)
	2	5/25 (20%)
	3	8/25 (32%)
TIMI flow post	0	0/25 (0%)
	1	0/25 (0%)
	2	1/25 (4%)
	3	24/25 (96%)

Expressed as n/N (%), mean (standard deviation), or median [Q1-Q3]. CABG: coronary artery bypass grafting; GPI: glycoprotein IIb/IIIa inhibitor; IU: international units; LAD: left anterior descending coronary artery; LCx: left circumflex coronary artery; PCI: percutaneous coronary intervention; RCA: right coronary artery; STEMI: ST-elevation myocardial infarction; TIMI: Thrombolysis In Myocardial Infarction

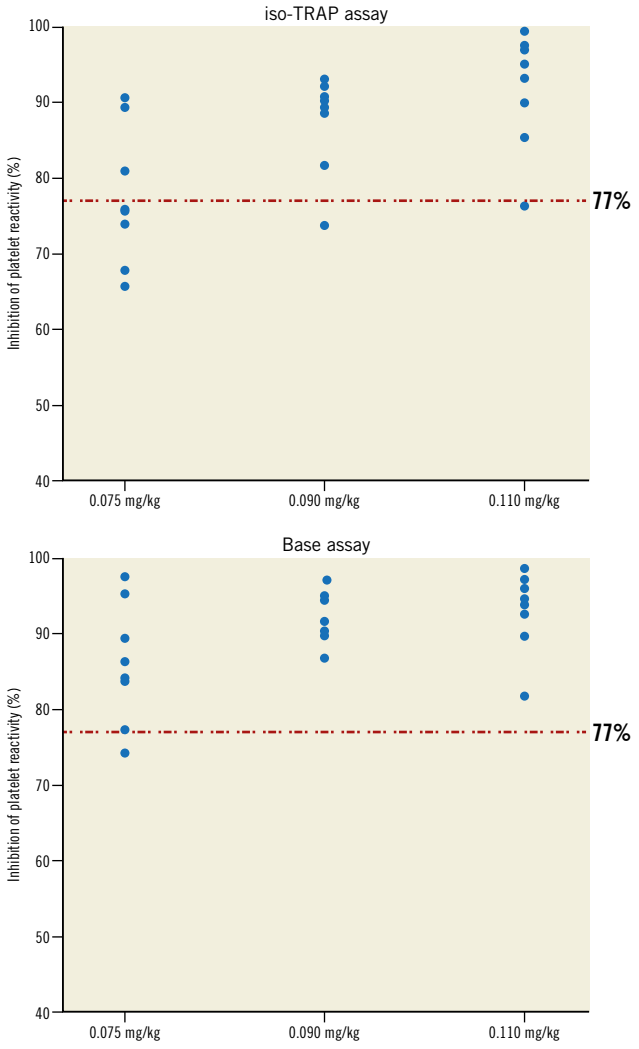


Figure 1. *Pharmacodynamic response at 15 minutes post dose to different doses of RUC-4. Inhibition of platelet reactivity, assessed by VerifyNow iso-TRAP (A) and Base (B, iso-TRAP + PAR-4) assays 15 minutes following subcutaneous injection of RUC-4 at 0.075, 0.090, and 0.110 mg/kg. PAR-4: protease-activated receptor 4; TRAP: thrombin receptor activating peptide*

TOLERABILITY

None of the patients included in the study developed thrombocytopaenia during the first 72 hours after the study drug administration. In the safety cohort, which included all patients receiving RUC-4 whether their data were evaluable or not, 25/27 (93%) reported at least one adverse event (AE) **(Table 2)**. The most common AEs were bruising at the RUC-4 injection site (41%) and vascular access-site haematoma or bruising (30%). The majority (69%) of these were rated as grade 1 (11/16) or grade 2 (3/16).

Eleven serious adverse events (SAEs) occurred in 8/27 patients (30%), of which 9/11 (82%) were judged not to be related to RUC-4. Two SAEs were assessed as probably related to RUC-4, but did not show a dose relationship, with one in a patient in the

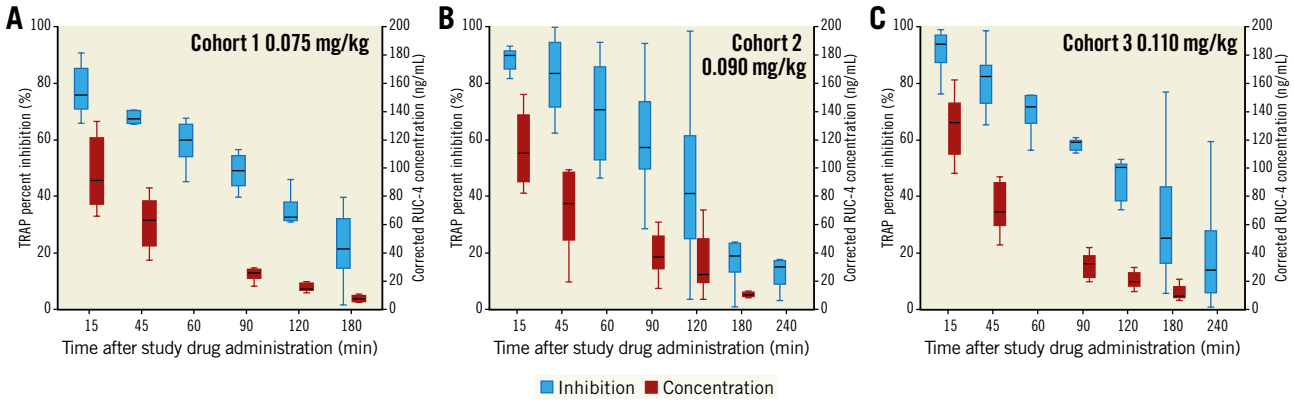


Figure 2. *RUC-4 pharmacokinetics and pharmacodynamics using the iso-TRAP assay. Platelet reactivity, assessed by VerifyNow and expressed as percent inhibition of the iso-TRAP assay (blue box plots) up to 180 min (cohort 1) or 240 min (cohorts 2 and 3), and plasma concentration of RUC-4 (red) up to 180 min (cohorts 1, 2, and 3) following subcutaneous injection of RUC-4 at 0.075 mg/kg (A), 0.090 mg/kg (B) and 0.110 mg/kg (C). Box plots represent the lower (25th) quartile, median, and upper (75th) quartile. IQR: interquartile range; TRAP: thrombin receptor activating peptide*

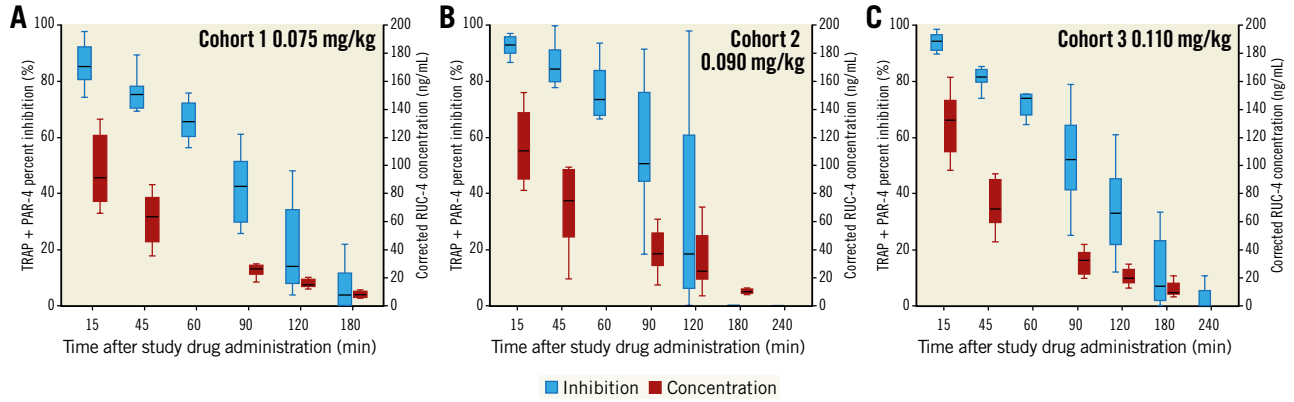


Figure 3. *RUC-4 pharmacokinetics and pharmacodynamics using the Base assay. Platelet reactivity, assessed by VerifyNow and expressed as percent inhibition of the Base assay (blue box plots) up to 180 min (cohort 1) or 240 min (cohorts 2 and 3), and plasma concentration of RUC-4 (red) up to 180 min (cohorts 1, 2, and 3) following subcutaneous injection of RUC-4 at 0.075 mg/kg (A), 0.090 mg/kg (B) and 0.110 mg/kg (C). Box plots represent the lower (25th) quartile, median, and upper (75th) quartile. IQR: interquartile range; PAR-4: protease-activated receptor 4; TRAP: thrombin receptor activating peptide*

0.075 mg/kg cohort and the other in the 0.110 mg/kg cohort. In both cases, the SAE was access-site bleeding from the radial artery (BARC type 3a and 3b). In 13 patients at least one BARC 1, and in two patients one or more BARC 2 bleeding was reported. One transient injection site reaction was reported, which was mild. There were no clinically meaningful changes in blood counts **(Table 3)**, or in kidney or liver function analytes. No deaths occurred during the study period.

Discussion

In the current Phase 2A study we analysed the PD and PK properties of RUC-4 in 27 patients with STEMI undergoing pPCI. The most important observations were: 1) RUC-4 rapidly achieved dose-dependent high-grade inhibition of platelet function, reaching a level that has been shown to correlate with improved clinical outcomes in patients treated with other GPI[10-12,20]; 2) the inhibition of platelet function declined after the 15-minute time point, reaching ~50% inhibition of platelet function between 90 and 120 minutes after administration in a dose-dependent manner; 3) the whole blood levels of RUC-4 correlated closely with the inhibition of platelet function and the correlation was consistent with the RUC-4 IC_{50} values for inhibiting platelet aggregation established *in vitro*[16,19]; 4) RUC-4 did not produce thrombocytopaenia in the first 72 hours post dose; and 5) the majority of bleeding/bruising complications were grade 1 or 2; there were two bleeding events classified as BARC 3a and 3b.

Table 2. Safety and tolerability of RUC-4.

Number of patients with	RUC-4 0.075 mg/kg	RUC-4 0.090 mg/kg	RUC-4 0.110 mg/kg	Total
Any AE	8/8 (100%)	7/9 (78%)	10/10 (100%)	25/27 (93%)
Grade 1	5/8 (63%)	5/9 (56%)	1/10 (10%)	11/27 (41%)
Grade 2	2/8 (25%)	0/9 (0%)	4/10 (40%)	6/27 (22%)
Grade 3	1/8 (13%)	2/9 (22%)	4/10 (40%)	7/27 (26%)
Grade 4	0/8 (0%)	0/9 (0%)	1/10 (10%)	1/27 (4%)
Grade 5	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Any AE related to study treatment	6/8 (75%)	5/9 (56%)	6/10 (60%)	17/27 (63%)
Grade 1	4/8 (50%)	5/9 (56%)	3/10 (30%)	12/27 (44%)
Grade 2	1/8 (13%)	0/9 (0%)	2/10 (20%)	3/27 (11%)
Grade 3	1/8 (13%)	0/9 (0%)	1/10 (10%)	2/27 (7%)
Grade 4	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Grade 5	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Any SAE	2/8 (25%)	2/9 (22%)	4/10 (40%)	8/27 (30%)
Grade 1	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Grade 2	1/8 (13%)	0/9 (0%)	0/10 (0%)	1/27 (4%)
Grade 3	1/8 (13%)	2/9 (22%)	3/10 (30%)	6/27 (22%)
Grade 4	0/8 (0%)	0/9 (0%)	1/10 (10%)	1/27 (4%)
Grade 5	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Any SAE related to study treatment	1/8 (13%)	0/9 (0%)	1/10 (10%)	2/27 (7%)
Grade 1	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Grade 2	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Grade 3	1/8 (13%)	0/9 (22%)	1/10 (10%)	2/27 (7%)
Grade 4	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
Grade 5	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)
AEs with fatal outcome/death	0 (0%)	0 (0%)	0 (0%)	0 (0%)
AEs of special interest				
Injection site reaction	0/8 (0%)	1/9 (11%)	0/10 (0%)	1/27 (4%)
Injection site bruising	3/8 (38%)	4/9 (44%)	4/10 (40%)	11/27 (41%)
Mild access-site bleeding	2/8 (25%)	1/9 (11%)	3/10 (30%)	6/27 (22%)
Severe access-site bleeding	1/8 (13%)	0/9 (0%)	1/10 (10%)	2/27 (7%)
Bleeding according to BARC classification				
1	4/8 (50%)	5/9 (56%)	5/10 (50%)	13/27 (50%)
2	1/8 (13%)	0/9 (0%)	1/10 (10%)	2/27 (7%)
3	1/8 (13%)	0/9 (0%)	1/10 (10%)	2/27 (7%)
4	0/8 (0%)	0/9 (0%)	0/10 (0%)	0/27 (0%)

Expressed as n/N (%). Subjects reporting more than one adverse event are counted only once using the highest grade. Grade 1: mild; Grade 2: moderate; Grade 3: severe or medically significant; Grade 4: life-threatening; Grade 5: death related to AE. AE: adverse event; BARC: Bleeding Academic Research Consortium; SAE: serious adverse event

Table 3. Haemoglobin and platelet count following RUC-4 administration.

	Haemoglobin in g/dL (mean, SD)			Platelet count as 109/L (mean, SD)		
	Pre-dose	24 hours post dose	72 hours post dose /discharge	Pre-dose	24 hours post dose	72 hours post dose /discharge
Cohort 1	14.04 (0.95)	13.82 (2.15)	14.10 (2.13)	226.38 (44.59)	211.13 (36.22)	214.88 (49.28)
Cohort 2	13.68 (1.17)	13.93 (1.46)	13.96 (1.14)	294.44 (74.60)	273.00 (71.72)	281.67 (79.81)
Cohort 3	14.31 (1.31)	13.29 (2.11)	13.75 (2.24)	283.44 (60.85)	238.13 (46.07)	229.00 (47.76)

SD: standard deviation

The earlier Phase 1 study of RUC-4 established its ability consistently to achieve high-grade inhibition of platelet function within 15 minutes of subcutaneous administration in healthy volunteers and in patients with stable CAD on aspirin[18]. The results of the current study are important since STEMI patients undergoing pPCI may differ in their response to RUC-4 from those healthy volunteers studied in Phase 1. They are concomitantly treated with aspirin, ticagrelor, and heparin. Also, pharmacokinetic responses may be altered by STEMI-induced selective shunting of blood to the vital organs, causing decreased perfusion of the skin and thus uptake of the investigational product. Despite these theoretical concerns, the PK/PD results in this study are similar to those in the Phase 1 study in healthy volunteers and patients with stable CAD on aspirin[18]. Patients with cardiogenic shock were excluded from our study, so it is unclear whether it will have an effect on RUC-4's PK/PD.

There is a need for drugs such as RUC-4 in the early treatment of patients with STEMI because the effect of oral $P2Y_{12}$ inhibition is delayed, especially in these STEMI patients[2]. Despite the faster platelet inhibition by either chewing or crushing oral $P2Y_{12}$ inhibitors[6-8], a gap of several hours in optimal platelet inhibition remains; until now, no study investigating oral $P2Y_{12}$ inhibition has shown a clinical benefit in STEMI patients[8].

In contrast, the use of GPI has been validated as an effective therapy for MI patients undergoing pPCI[23], and in meta-analyses of early administration in the ambulance or emergency department has been shown to be superior to in-hospital administration in reperfusing the target artery, improving ST-segment resolution, left ventricular function, and mortality[12,13]. Despite these data, GPI are not routinely administered in the ambulance or emergency department, in part because they require IV administration as a bolus and continuous infusion controlled by a pump. Also, the reduction of thrombotic events was counterbalanced by more severe bleeding in patients treated with GPI[23], although some of this bleeding has been ameliorated by the increased use of radial artery access[24].

In this study, two severe access-site bleedings were judged by the PCI operators to be most likely due to catheter-based trauma to the proximal radial artery. As it is a potent antiplatelet agent, the investigators judged the haematomas probably to be related to RUC-4 treatment; however, it was difficult to assess its precise contribution since, in addition to the presumed arterial injury, the patients were treated with aspirin, heparin, and ticagrelor, all of which are also known to increase bleeding. Overall, the study was not designed or powered to evaluate safety.

In addition, all of the current GPI are associated with thrombocytopaenia in a small percentage of patients (0.5%-2%)[15]. Since RUC-4 is unique among small molecule GPI in not inducing the receptor to undergo a major conformational change that has been implicated in the development of thrombocytopaenia[25], it is possible that RUC-4 may be associated with fewer episodes of thrombocytopaenia than current GPI. Additional studies will be required to assess this hypothesis.

The only approved parenteral $P2Y_{12}$ inhibitor, cangrelor, is effective in achieving rapid platelet inhibition[26] but, like currently available GPI, it requires continuous IV administration, whereas RUC-4 is administered by a single subcutaneous dose. Moreover, even the most potent $P2Y_{12}$ inhibitors are less effective in inhibiting platelet function than GPI because they only inhibit one activation pathway (ADP-initiated), whereas GPI block the final pathway downstream of all of the activation pathways[9,27]. Furthermore, cangrelor is not currently recommended for pre-hospital use; rather it is used in patients who do not receive a $P2Y_{12}$ inhibitor before the procedure or in those considered unable to absorb oral agents[28].

The antiplatelet effects of RUC-4 wear off rapidly, reaching about 50% inhibition between 90 and 120 minutes after administration. This reduces the time during which there is high-grade inhibition of platelet function and thus should reduce the risk of haemorrhage relative to the current agents that inhibit the receptor for longer periods of time. The primary goal with RUC-4 is to initiate immediate reperfusion as rapidly as possible and prevent re-occlusion before and during the pPCI. In the current study, the time from administration of aspirin, ticagrelor, and heparin to pPCI was 50±16 minutes, a time period during which RUC-4 produced greater than 50% inhibition of platelet function. Thus, the optimal time for the effects of RUC-4 to dissipate will depend on the local metrics with regard to performing the PCI. Our data show that the higher doses of RUC-4 not only achieve greater inhibition of platelet function at 15 minutes, but also achieve longer duration of inhibition of platelet function. Thus, if it is deemed more desirable to prolong the effect of RUC-4, this can be accomplished by increasing the dose. This will be studied in the upcoming Phase 2B CELEBRATE trial.

Another experimental subcutaneously administered $P2Y_{12}$ inhibitor, selatogrel, has been investigated in patients with MI[29]. Forty-seven patients were randomised to a single subcutaneous dose prior to PCI. Selatogrel inhibited the VerifyNow $P2Y_{12}$ assay, but no data were provided on its ability to inhibit TRAP-induced platelet aggregation. Selatogrel's effects last for at least eight hours, which will also affect its efficacy and safety profile. Data from other $P2Y_{12}$ antagonists, including the potent prasugrel, indicate that doses that cause near-complete inhibition of the $P2Y_{12}$ assay do not inhibit the Base channel assay[30]. In sharp contrast, within 15 minutes, RUC-4 inhibited the Base channel assay on average by more than 90%. Clinical studies are required to assess whether the enhanced *in vitro* antiplatelet effects of RUC-4 will translate into improved clinical outcomes. It is also possible that combining RUC-4 and selatogrel may be beneficial.

Limitations

Our study has several limitations. First, it had an open-label design without a placebo, but this design is unlikely to influence the PD and PK data of our study. Second, as currently configured, the iso-TRAP and Base channel assay data are not reported by the VerifyNow instrument and so they needed to be calculated from the raw data obtained from the instrument. Thus, they are currently not commercially available. VerifyNow assays have the advantage

of being automated and so are much easier to perform in the emergency setting than the labour-intensive and difficult-to-standardise LTA, the current gold standard of platelet function testing. Since *in vivo* antithrombotic effects and clinical outcomes have correlated closely with ADP-induced LTA[20], we calibrated these assays against ADP-induced LTA **(Supplementary Appendix 2)** and found that RUC-4 achieves dose-dependent inhibition of platelet function comparable to the LTA standard of 80% within 15 minutes after subcutaneous administration.

The timing of subcutaneous injection and start of the PCI does not fully resemble the expected use of RUC-4 in clinical practice. To maintain control over the administration of RUC-4 until more is known about its effects in STEMI, in the current design, the aspirin, ticagrelor, and heparin were administered about 50 minutes before RUC-4 and the PCI was initiated immediately after the administration of RUC-4. The future intended application of RUC-4 would have it administered at the same time as the aspirin, ticagrelor, and heparin, and about an hour before the PCI. Thus, the peak of RUC-4's antiplatelet effect in this study occurred at a time point when ticagrelor's effect was already beginning to become manifest, potentially contributing to the risk of bleeding. In addition, RUC-4 had little time in which to achieve its antithrombotic effects prior to PCI and a greater chance of causing bleeding during PCI. This may be responsible for some of the bleeding events. When used according to the future intended application, the antiplatelet effects of RUC-4 will be less prominent at the time of vascular closure, and thus will probably contribute less to access-site bleeding complications.

Conclusions

In this phase 2A study in patients with STEMI, a single subcutaneous dose of RUC-4 induced dose-dependent maximal platelet function inhibition within 15 minutes, with 50% return of platelet function roughly over 90 minutes, increasing with higher doses. The results of this study warrant further research on pre-hospital treatment with RUC-4 as a bridge to onset of oral antiplatelet agents.

Impact on daily practice

RUC-4 provides fast and potent platelet inhibition by a single subcutaneous injection. Thus, it may offer advantages compared to intravenous GPI and (oral) P2Y12 inhibitors for first-point-of-care treatment of STEMI. Its short duration of effect is designed to initiate target vessel reperfusion medically en route to PCI, while limiting the risk of bleeding during and after the procedure. The results of this study warrant further research on pre-hospital treatment with RUC-4. An upcoming Phase 2B trial will assess the efficacy and safety of RUC-4 administration to STEMI patients in the ambulance.

Acknowledgements

The authors thank all patients, study investigators, study staff, CCL staff and nursing teams for their participation in this study. The authors acknowledge E. Kolkman, an employee of Diagram B.V., for providing statistical support during manuscript development. The authors also acknowledge the contribution of F.W.A. Verheugt for his independent role in the Safety Review Committee.

Funding

This study was supported by a grant and materials provided by CeleCor Therapeutics.

Conflict of interest statement

M. Gibson receives research support from Johnson & Johnson. He receives consulting support from AstraZeneca, Johnson & Johnson, and Janssen & Bayer. C. Granger reports consultancy or research funding from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, CeleCor, Daiichi Sankyo, Janssen, Pfizer, Novartis, NHLBI, and the FDA. O. Bentur reports research fees from CeleCor and Pulmoquine Therapeutics. B.S. Coller receives royalties from the sales of abciximab (Centocor/Janssen) and the VerifyNow assays (Accumetrics/Instrumentation Laboratories). He is also an inventor of RUC-4, a founder and equity holder in CeleCor, and a consultant to CeleCor. B.S. Coller served as a non-voting scientific consultant to the Safety Review Committee and was supported in part by grant 19278 from the National Heart, Lung and Blood Institute and Clinical and Translational Science grant UL1 TR001866 from the National Center for Advancing Translational Science. A.W.J. van 't Hof reports institutional unrestricted grants from Medtronic, Boehringer Ingelheim, AstraZeneca and Abbott, not related to this work. J.M. ten Berg reports lecture or consultancy fees from AstraZeneca, Eli Lilly, Daiichi Sankyo, The Medicines Company, AccuMetrics, Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, Bayer, Ferrer, and Idorsia. He received institutional research grants from ZonMw, and AstraZeneca. The other authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Appendix 1. Study protocol.

Supplementary Appendix 2. Correlation between LTA and VerifyNow iso-TRAP assays.

The supplementary data are published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-21-00287

PROTOCOL CEL-02

V1.4, Protocol amendment 2.0

27 August 2020

A Phase 2 open label study to assess the pharmacodynamic and pharmacokinetic properties of a single subcutaneous injection of RUC-4 in patients with a ST-elevation myocardial infarction presenting to the cardiac catheterization lab with planned primary coronary angioplasty

Protocol Number	**CEL-02**
Sponsor	**CeleCor Therapeutics, Inc.** **1155 Camino Del Mar Suite 481** **Del Mar, CA 92014 (USA)** **Robert S. Hillman, PhD (President and Chief Executive Officer)** **Telephone: 858-777-9750**
Principal Investigator	**Dr. J.M. Ten Berg** **St. Antonius hospital** **Koekoekslaan 1** **3435 CM Nieuwegein (NL)**
Co-Principal investigator	**Prof. A.W.J. Van 't Hof** **Maastricht University Medical Centre^{+} (MUMC^{+})** **P. Debyelaan 26** **6229 HX Maastricht (NL)**
CRO	**Diagram B.V.** **Dokter Stolteweg 96** **8025 AZ Zwolle (NL)**
Safety Review Committee	**For Members see Safety Review Committee Charter**
Medical Monitor	**R. Lobatto**
Import, release and distribution study drug	**Ace Pharmaceuticals BV** **Zeewolde (NL)**
Time schedule	**First patient in (FPI): 25 May, 2020** **Last patient in (LPI): 1 October, 2020** **Last patient out (LPO): 31 October, 2020**

PROTOCOL SIGNATURE SHEET

This study will be conducted according to the principles of Good Clinical Practice (GCP) as described in International Council for Harmonisation (ICH) guidelines, including the archiving of essential documents.

CONFIDENTIALITY STATEMENT

The information contained in this document and all information provided to you related to CEL-02 are the confidential and proprietary information of CeleCor Therapeutics, Inc. (CeleCor) and except as may be required by federal, state, or local laws or regulations, may not be disclosed to others without prior written permission of CeleCor. However, the Principal Investigator (PI) may disclose such information to supervised individuals working on CEL-02, provided such individuals agree to be bound to maintain the confidentiality of such information.

Title: A Phase 2 open label study to assess the pharmacodynamic and pharmacokinetic properties of a single subcutaneous injection of RUC-4 in patients with ST-elevation myocardial infarction presenting to cardiac catheterization lab with planned primary coronary angioplasty

Protocol Version: 1.4, protocol amendment 2.0

Protocol Date: 27 August, 2020

I have received and read the current edition of the Investigator's Brochure for RUC-4. I have read this version of the CEL-02 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Principal Investigator

HISTORY OF CHANGES

REVISION	Change	Release date
1.0	**Initial release**	**14 November 2019**
1.1	**- Medical monitor** **- Extension rationale study design** **- Deleted two endpoints:** **- ADP-induced platelet aggregation (%) assessed by VerifyNow PRUTest** **- TIMI frame count** **- Minor textual changes**	**15 January 2020**
1.2	**- Additional telephonic visit at 15 days ± 2 days** **- Total study duration has been extended** **- Safety is part of primary endpoint** **- All lab as part of standard of care will be collected** **- ACT will be measured after any heparin dose adjustment** **-Causality assessment has been updated**	**13 February 2020**
1.3	**Protocol amendment:** **- Additional PD measurement at 240 minutes if the dose of the study drug is increased (cohort 2 and/or 3)** **- Update timelines** **- Add exclusion criteria regarding COVID-19 infection** **- Definition of causal relationships with study drug and PCI procedure has been added** **- ≥77% instead of 90% inhibition of the VerifyNow PRUTest**	**15 April 2020**
1.4	**Protocol amendment:** **-Additional heparin administration if ACT is <200 seconds instead of <250 seconds** **-Adapt exclusion criteria regarding COVID-19 infection** **-Adapt inclusion criteria regarding persistent vs ongoing ST elevation** **-Remove exclusion criterium regarding de novo AF** **-Updated time schedule** **-VerifyNow measurement now includes additional BASE channel; P2Y12 Test cartridges instead of PRU; update to Rationale and Dose selection section accordingly**	**27 August 2020**

	-addition of optimal VerifyNow sample if required until return toward Baseline is observed	

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ADP	Adenosine diphosphate
AE	Adverse event
ACT	Activated Clotting Time
AUC	Area under the curve
AUC(0-t)	Partial area under the concentration-time curve to time (where t may be 15, 180 m or other as appropriate) will be calculated using the linear trapezoidal rule for increasing concentrations and the log trapezoidal rule for decreasing concentrations
AUC(0-last)	Area under the blood concentration-time curve from time zero to time of last quantifiable concentration calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations
AUC(0-∞)	Area under the blood concentration-time curve from time zero extrapolated to infinity. AUC = AUC(0-last) + C(last) / lambda_z
ALT	Alanine aminotransferase
BARC	Bleeding Academic Research Consortium
BED	Biologically effective dose
BID	Twice daily
BLQ	Below the level of quantification
BMI	Body mass index
BSEP	Bile salt export pump
BUN	Blood urea nitrogen
CA	Competent Authority
CAD	Coronary artery disease
CEC	Clinical Events Committee
CL	Clearance = Dose/AUC(0-∞)
CCL	Cardiac catheterization lab
C_{max}	Maximum blood concentration
CNS	Central Nervous System
CRA	Clinical Research Associate

CTM	Clinical Trial Monitor
CYP	Cytochrome P450
DOAC	Direct oral antagonist
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
e-CRF	Electronic case report form
EMS	Emergency Medical System
EudraCT	European drug regulatory affairs Clinical Trials
FDA	Food and Drug Administration
FN	False negative
FP	False positive
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hERG	Human ether-à-go-go-related gene
ICH	International Council for Harmonisation
ISTH	International Society on Thrombosis and Haemostasis
IV	Intravenous(ly)
k_{el}	Terminal phase elimination rate constant
Labda_z	Terminal phase rate constant
LLOQ	Lower limit of quantification
LTA	Light transmission platelet aggregation
Max	Maximum
MEDDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
MIDAS	Metal ion-dependent adhesion site
Min	Minimum
MN	Micronuclei
MTD	Maximum tolerated dose
NHP	Nonhuman primate
NOAEL	No-observed-adverse-effect-level
OHCA	Out of hospital cardiac arrest

PCE	Polychromatic erythrocyte(s)
PCI	Percutaneous coronary intervention
PD	Pharmacodynamic(s)
PGE1	Prostaglandin E1
PI	Principal Investigator
PK	Pharmacokinetic(s)
PPACK	D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone
PRU	P2Y12 reaction unit
PT	Prothrombin time
Q	Quartile
RBC	Red blood cell
SAE	Serious adverse event
SC	Subcutaneous(ly)
SOC	System organ class
SPC	Summary of Product Characteristics
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator
STEMI	ST-elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reactions
$T_{1/2}$	Half-life (ln2/ lambda_z)
TEAE	Treatment-emergent adverse event
TIMI	Thrombolysis in Myocardial Infarction
T_{max}	Time of maximum blood concentration
TN	True negative
TP	True positive
TRAP	Thrombin receptor activating peptide
US	United States
UIP	University of Iowa Pharmaceuticals
VD	Volume of distribution = Dose/lambda_z* AUC(0-∞)
VKA	Vitamin K antagonist
WBC	White blood cell
WMO	Medical Research Involving Human Patients Act

SUMMARY

Rationale:

RUC-4 is a novel, promising and fast acting (5-15 minutes) αIIbβ3 receptor antagonist with a high-grade inhibition of platelet aggregation (≥80%) shortly after subcutaneous administration. For patients with ST-elevation myocardial infarction (STEMI) early treatment with RUC-4 could be beneficial by improving initial patency of the infarct related vessel and by minimizing thrombotic occlusions, thus improving both coronary artery and myocardial microvascular blood flow, possibly resulting in a decrease in infarct size and a reduction in complications of STEMI.

Objective:

The main objective is three-fold:

- To assess the pharmacodynamic (PD) properties of a single subcutaneous injection of RUC-4 in STEMI patients presenting to the cardiac catheterization lab (CCL) with planned primary coronary angioplasty
- To assess the pharmacokinetic (PK) properties of a single subcutaneous injection of RUC-4 in STEMI patients presenting to the CCL with planned primary coronary angioplasty
- To assess safety and tolerability of RUC-4

Study design:

This is an open-label, Phase 2 single center study.

Sample size:

The estimated number of patients to be enrolled is 30, allocated in up to three cohorts of eight at an initial starting dose of 0.075 mg/kg. Each cohort will consist of eight evaluable patients. If a patient is not evaluable, but has been enrolled (e.g. baseline PD and/or PK assessments cannot be performed or the patient prematurely discontinues for whatever reason before the study procedures are performed), extra patients may be enrolled. At the completion of dosing for the first cohort, the Safety Review Committee (SRC) will make the determination to increase, decrease or maintain the same dose as outlined in the SRC charter.

Study population:

Inclusion criteria:

1. Patients with STEMI, presenting with persistent chest pain (>30 min) and ≥1 mm ST-segment elevation in two adjacent electrocardiograph leads, with >6 mm cumulative ST-segment deviation, in whom the total duration of symptoms to first intracoronary device deployment (excluding a wire) is anticipated to be within 6 hours
2. Adult males and females 18 years of age or older
3. Females must be non-pregnant, non-lactating, and of non-childbearing potential (postmenopausal or surgically sterilized) by history and review of medical record
4. Weight (by history) of between 52 and 120 kg
5. Written informed consent (following short-form of the informed consent form at CCL)

Exclusion criteria:

1. High probability in the opinion of the cardiologist that current STEMI is caused by stent thrombosis and the previous PCI related to this stent thrombosis is <1 month
2. High suspicion of type II MI
3. Current active coronavirus disease 2019 (COVID-19) infection (criteria according to local guidelines).
4. Out of hospital cardiac arrest (OHCA)
5. Therapy resistant cardiogenic shock (systolic blood pressure ≤80 mm Hg for >30 minutes)
6. Persistent severe hypertension (systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg)
7. Known severe liver disease
8. Known history of severe renal dysfunction (glomerular filtration rate <30 mL/min or serum creatinine >200 mmol/L [>2.5 mg/dL])
9. Known left bundle branch block
10. Requirement of oral anticoagulation (Vitamin K antagonists {VKA} or direct oral antagonists {DOACs})
11. Current treatment with αIIbβ3 receptor antagonist (other than RUC-4)
12. Coagulation abnormality, known bleeding disorder, or history of documented prior hemorrhagic or thrombotic stroke within the past 6 months
13. History of upper or lower GI bleeding within the past 6 months
14. Known clinically important anemia
15. Known clinically important thrombocytopenia (platelet count of less than 150,000/µL)
16. Known history of allergy to any of the ingredients in the RUC-4 formulation (i.e., acetate buffer, sucrose)
17. Major surgery within the past 6 months

18. Life expectancy of less than 6 months
19. Any clinically significant abnormality identified prior to enrollment that in the judgment of the Investigator would preclude safe completion of the study
20. Unwillingness or inability to comply with the requirements of this protocol including the presence of any condition (physical, mental, or social) that is likely to affect the patient's ability to comply with the study protocol

Intervention:

All patients will receive a single subcutaneous dose of RUC-4 in the CCL before coronary angiography/percutaneous coronary intervention.
Dose escalation as defined in the SRC charter can be performed twice.

Main study parameters/endpoints:

The main study parameter/endpoints:

- Inhibition of thrombin receptor activating peptide (TRAP)-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and at 240 minutes after administration of RUC-4 (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3)
- RUC-4 concentration (ng/mL) versus time profiles (at baseline and at 15, 45, 90, 120 and 180 minutes after administration of RUC-4) and associated PK parameters
- Safety and tolerability parameters at baseline and at hospital discharge

1. INTRODUCTION AND RATIONALE

1.1 Background

RUC-4 is a novel small molecule inhibitor of the platelet αIIbβ3 receptor specifically designed for first medical contact therapy of ST-elevation myocardial infarction (STEMI). Treating patients soon after the onset of symptoms provides the greatest opportunity to diminish both short-term and long-term morbidity and mortality, including heart failure (1-4). The incidence of hospital admissions for STEMI varies among the countries, however it is anticipated to be 66 patients/100,000/year (5). The mortality rate remains substantial with approximately 12% mortality at six months (6) and with an even higher risk in high risk patients (7). Myocardial infarction (MI) is thought to be a major contributor to the incidence of out-of-hospital deaths due to cardiac disease annually. RUC-4 is being designed to be easily administered subcutaneously (SC) by an auto-injector to facilitate its use as the first point of medical contact treatment for STEMI.

Treatment of MI with αIIbβ3 Antagonists

The use of αIIbβ3 antagonists has been validated as an effective therapy of MI for patients undergoing percutaneous coronary interventions (PCI). Treatment with one of the three currently available agents (abciximab, tirofiban, eptifibatide) has been shown to result in an approximately 20% reduction in mortality and an approximately 33% reduction in death or reinfarction at 30 days after treatment (8). Early treatment of MI with αIIbβ3 antagonists at first medical contact (i.e., by Emergency Medical System [EMS] personnel or personnel in emergency departments of either ‘spoke’ hospitals or PCI-capable hospitals) compared to catheterization lab treatment has been associated with increased pre-procedure blood flow in the target coronary artery using the Thrombolysis in Myocardial Infarction (TIMI) scale and indices of myocardial perfusion, smaller infarcts, fewer early and late complications of MI, and reduced mortality (1-3). The improvement in outcome correlates with the time at which the drugs were administered after the onset of symptoms (2, 9). Despite these data, αIIbβ3 antagonists are not routinely administered at first medical contact, in part because they require intravenous (IV) administration of a bolus dose followed by a continuous infusion regulated by an infusion pump. In addition, all of the agents are associated with thrombocytopenia in a small percentage of patients (0.5%-2%), with abciximab associated with the highest frequency (10). RUC-4 is being developed to facilitate pre-hospital and emergency department therapy at the earliest time point, thus maximizing the chance of preserving the cardiac muscle. RUC-4 is differentiated from current αIIbβ3 antagonists

because it is based on newer information on the receptor structure and is specifically designed to facilitate early administration (11-12). RUC-4 inhibits ligand binding to αIIbβ3 by binding to both the αIIb and β3 subunits and displacing the $Mg2^{+}$ metal from the ion-dependent adhesion site (MIDAS) required for ligand binding; this locks the β3 subunit of the receptor in its inactive conformation. This may decrease the likelihood of developing thrombocytopenia because data indicate that much of the thrombocytopenia caused by the current αIIbβ3 antagonists is due to the presence of antibodies to conformations of the receptor induced by the binding of the drugs (10). To facilitate administration by EMS and emergency department personnel, RUC-4 was designed for SC administration. In addition, RUC-4 is designed to have high aqueous solubility, so that it could potentially be administered in a small volume by auto-injector. The rapid onset of RUC-4's antiplatelet effect (within 15 minutes) and its offset 1-2 hours after administration are designed to provide optimal cardiac protection until PCI can be performed without hampering the receiving hospital's ability to individualize therapy, including cardiac surgery.

Pharmacology and Pharmacokinetics Studies

The potency of RUC-4 was assessed by measuring its antiplatelet activity ex vivo by its ability to inhibit platelet aggregation induced by adenosine diphosphate (ADP) (12). Its selectivity was established by its lack of inhibition of the most closely related integrin receptor, αVβ3, as judged by the adhesion of cells expressing αVβ3 to immobilized vitronectin (12). The pharmacodynamic (PD) profile of RUC-4 was assessed in a number of different animal models. As a prelude to clinical studies, the pharmacokinetic (PK) of RUC-4 was investigated in mice and NHPs. RUC-4 is rapidly absorbed following subcutaneous injection in mice and monkeys, with time to maximum blood concentration (T_{max}) approximately 15 min post dosing. Preliminary estimates indicate that RUC-4 has high systemic bioavailability (essentially 100%). The drug has a half-life (T1/2) value of approximately 0.1 to 0.5 hours after IV administration and approximately 0.4 to 0.6 hours after SC administration. RUC-4 is metabolically stable when incubated with hepatocyte microsomes from mice, monkeys, and humans (T1/2 > 120 minutes), indicating that it is not a substrate for cytochrome P450 (CYP)-mediated metabolism in vitro. Studies indicated that CYP-mediated drug-drug interactions are considered unlikely. RUC-4 had no significant activity against a broad panel of uptake and efflux transporters in vitro except for MATE1 and bile salt export pump (BSEP). Because MATE1 may decrease the renal excretion of certain drugs such as metformin, patients in Part 2 of this study who have taken metformin less than 24 hours before enrollment will be excluded from the study. Liver function will be carefully monitored clinically given the effect of RUC-4 on BSEP-mediated transport. However, given

the intended single dose use of RUC-4 and the lack of hepatotoxicity observed in pre-clinical studies, the risk of hepatic toxicity by this mechanism is considered low.

Toxicology, Safety Pharmacology, and Genotoxicity Studies

Toxicology, safety pharmacology, and genotoxicity studies were conducted to evaluate the nonclinical safety profile of RUC-4. The Safety Pharmacology A panel of safety pharmacology studies were conducted evaluating the effect of RUC-4 on cardiovascular, respiratory, and Central Nervous System (CNS) safety. In a radiotelemetry study conducted in conscious male cynomolgus monkeys, RUC-4 (100 mg/kg dose level) caused a slight decrease in body temperature (up to 0.71°C decrease compared to control), a transient decrease in heart rate (up to 16.4% mean decrease compared to control), and swelling near or around the injection site. In a CNS safety pharmacology study evaluating the gross behavioural, physiological, and neurological state of male CD-1 mice, lethality was observed in 2 of 6 mice administered the highest dose of 200 mg/kg and varying degrees of tremors, walking low on limbs, decreased cutaneous flow, and blue skin on the dorsal thoracic area were noted at dose levels of RUC-4 ≥ 50 mg/kg. The in vitro effects of RUC-4 and its metabolite des-glycine RUC-4 on ionic currents in voltage-clamped human embryonic kidney cells (HEK-293) that stably express the human ether-à-go-go-related gene (hERG) were determined. The IC_{50} values for the inhibitory effect of RUC-4 and des-glycine RUC-4, respectively, on hERG potassium current were estimated to be > 260 µM and > 30 µM. Thus, neither RUC-4 nor its metabolite, desglycine RUC-4 had an effect on the hERG channel current, at the concentrations tested. The standard panel of assays were applied to evaluate the genotoxic potential for RUC-4. RUC-4 was evaluated for mutagenic activity in the in vitro Salmonella E. coli/mammalian microsome reverse mutation assay. These studies support the conclusion that RUC-4 is negative for mutagenic activity. In addition, studies employing the in vitro micronucleus assay to evaluate if RUC-4 interferes with normal mitotic cell division were shown to be negative. RUC-4 was evaluated to assess for the potential to induce micronuclei (MN) in polychromatic erythrocytes (PCEs) in mouse bone marrow at 24 and 48 hours following SC (twice daily [BID]) injection in mice. At doses of up to 300 mg/kg/day, RUC-4 did not produce statistically significant or dose-dependent increases in the

% MN-PCEs. Thus, RUC-4 was negative for clastogenic activity and/or disruption of the mitotic apparatus under the conditions of this assay. Toxicology A nonclinical toxicology program conducted for RUC-4 under Good Laboratory Practice (GLP) conditions included studies performed in rodent (mouse) and non-rodent (cynomolgus monkey) species. For both the mouse and monkey, RUC-4 was administered by SC injection twice in a single day separated by approximately 4 hours. In both species, the injection sites and kidney were target organs after RUC-4 administration (Day 2). The mechanism(s) of the injection site and

kidney findings are uncertain. The kidney changes were limited to the highest dose groups evaluated, 200 mg/kg/day (100 mg/kg/dose) in both species and the pattern of alterations in the kidney were comparable (degeneration or necrosis of renal tubules); however, lesions were limited to the high-dose females in the mouse study. In monkeys, where the kidney changes were more severe and considered adverse at 200 mg/kg/day (100 mg/kg/dose), renal biomarker changes (increased urea nitrogen, creatinine, and phosphorus) were noted. In both species, the renal tubular degeneration and/or necrosis resolved during the recovery period (Day 15). In monkeys, there was lower erythrocyte mass (red blood cell [RBC], hemoglobin, and hematocrit) that was associated with clinical, gross, and/or microscopic hemorrhage in individual animals. The magnitude of the decrease in erythrocyte mass in the 20 mg/kg/day (10 mg/kg/dose) female group and the 200 mg/kg/day (100 mg/kg/dose) male and female groups along with the accompanying clinical, gross, and/or microscopic changes was considered adverse. There was no evidence microscopically of bone marrow injury and the erythrocyte mass resolved at the end of the recovery period in the 20 mg/kg/day female group and in one male from the 200 mg/kg/day male group. Increased reticulocyte counts indicating a regenerative response to the hemorrhage were observed at the recovery necropsy in the 20 mg/kg/day female group and the 200 mg/kg/day male and female groups. The hemorrhage and subsequent decrease in erythrocyte mass were considered secondary to the pharmacology of RUC-4 and likely was exaggerated in affected monkeys and likely due to required procedural-related handling. The injection site observations had a pattern consistent with a local response to RUC-4 and were limited to the SC space at Day 2. By Day 15, this response was largely resolved. The pattern of localized RUC-4 injection site response was similar to that reported in reviews of local anesthetics (lidocaine, bupivacaine, procaine, others) injected SC in preclinical studies and 30 vaccine preclinical toxicology studies: degeneration/necrosis of the panniculus carnosus, mixed cell inflammation, and hemorrhage (13). For both the mouse and monkey, the degeneration/necrosis observed in the SC injection site was generally restricted to the panniculus carnosus muscle. Sections of skin examined at the periphery of the injection site area had marked reductions in severity and incidence of the injection site observations, supporting that the injection site reaction was a localized response. No other muscles, including the underlying muscle of the dorsoscapular region, were involved. Muscle injury at other sites in the monkey occurred sporadically in 20 and 200 mg/kg/day females but was secondary to bruising as a result of physical restraint and the pharmacology of RUC-4. There was no evidence of RUC-4-related cardiac injury microscopically or RUC-4-related changes in Troponin I concentration. In the mouse, the no observed adverse effect-level (NOAEL) was considered to be 25 mg/kg/dose (50 mg/kg/day). In the cynomolgus monkey, the NOAEL for systemic toxicity was considered

to be 20 mg/kg/day free base equivalent for males and 2 mg/kg/day free base equivalent for females.

Clinical Experience with RUC-4

The Phase 1 clinical study CEL-01 (NCT03844191) assessed the safety, tolerability, and PK and PD of escalating doses of RUC-4 (until a weight-adjusted biologically effective dose {BED} or maximum tolerated dose {MTD} was identified) administered SC in healthy volunteers and in patients on aspirin with stable coronary artery disease (CAD). Enrollment into the dose escalation phase has been completed, and the dose expansion phase is ongoing in subjects on aspirin with stable CAD. Clinical safety data, including Adverse Events (AEs), bleeding events, and injection site reactions, and platelet aggregation results continue to be closely monitored by a Safety Review Committee (SRC). Safety and PD data from the Phase 1 CEL-01 study are summarized below.

Three single RUC-4 doses have been tested 0.04 mg/kg, 0.05 mg/kg, and 0.075 mg/kg in:

- 14 healthy volunteers [RUC-4 n=12, 0.05 mg/kg (n=6) and 0.075 mg/kg (n=6), placebo n=2]
- 30 subjects on aspirin with stable CAD [RUC-4 n=26, 0.04 mg/kg (n=2), 0.05 mg/kg (n=6), and 0.075 mg/kg (n=18), placebo n=4]

RUC-4 at a 0.075 mg/kg dose provides rapid (<15 minutes), potent (>80% inhibition of ADP-induced platelet aggregation), and short-term half-life (<2 hours) inhibition of platelet aggregation after subcutaneous treatment. RUC-4 was well-tolerated by healthy volunteers and patients with stable CAD on aspirin. All subjects have completed the study. The data analysis shows:

- No serious adverse events (SAEs), the majority of AEs were mild in intensity and have resolved or are resolving, no AEs led to study discontinuation
- Bleeding events were uncommon, were mild (modified BARC type 1) and were limited to the injection site (n=3)
- The majority of injection site reactions, including bruising, were mild and resolved
- No drug-related changes in laboratory values, including platelet counts, nor post-dose ECGs
- Daily aspirin (81 mg or 325 mg) use did not appear to significantly affect RUC-4 PK or PD, nor did it increase bleeding

1.2 Rationale for the Phase 2 study

1.2.1 Rationale for study design

For patients with STEMI, early treatment with RUC-4 could be beneficial by improving initial patency of the infarct related vessel and by minimizing thrombotic occlusions during the expected PCI, thus improving both coronary artery and myocardial microvascular blood flow, possibly resulting in a decrease in infarct size and a reduction in complications of STEMI. Therefore, the Phase 2 study is designed to assess PD and PK properties of the weight-adjusted dose of RUC-4 (mg/kg) required to achieve 77% or greater inhibition of thrombin receptor activating peptide (TRAP) induced platelet aggregation within 15 minutes of SC administration of RUC-4 with return toward baseline values within 4 hours in STEMI patients presenting to the cardiac catheterization lab (CCL).

1.2.2 Rationale for dose and schedule

The rationale for dose selection and dose escalation is based in part on the NOAEL for systemic toxicity of 20 mg/kg and 2 mg/kg RUC-4 free base equivalent in male and female Non-human primates (NHPs), respectively.
In the CEL-01 trial, a RUC-4 dose of 0.075 mg/kg achieved the biologically effective dose (BED) goal of 80% inhibition of ADP-induced platelet aggregation; although based on the toxicology data in female NHP, a dose of up to 2.4 mg/kg was considered safe. Dose escalation beyond the BED was not done in the CEL-01 study, as healthy volunteers and stable patients on aspirin would not receive a possible direct benefit from participating, while there are documented benefit(s) of glycoprotein αIIbβ3 inhibitor in STEMI patients (14). The Phase 1 study assessed the effects of RUC-4 using 80% of ADP-induced platelet aggregation as the target. Unlike the Phase 1 study, in the Phase 2A study (CEL-02), it is anticipated that ticagrelor will be administered to all STEMI patients as standard of care prior to RUC-4 administration. In this study the VerifyNow P2Y12 reaction unit (PRU)Test will be used. The VerifyNow P2Y12Test has three assay channels that can measure platelet aggregation, ADP (+prostaglandin E1 {PGE1}) iso-TRAP, and iso-TRAP (+PAR-4 inhibitor). Ticagrelor

- effects the ADP induced platelet aggregation,
- has minimal effect on the iso-TRAP channel early on & variable effect after,
- and no effect on the P2Y12Test BASE assay

The iso-TRAP channel will be used to monitor the initial (15, 30 minute) response to RUC-4; in order to more easily compare the Phase 1 and Phase 2 data. However, the terminal PD of

RUC-4 (after 60 minutes) will be measured by the BASE channel, to remove any variable contribution of ticagrelor to platelet inhibition and estimate the offset of RUC-4 . At baseline, the ADP+PGE1 assay will allow assessment of the impact of ticagrelor. In the Phase 1 study, D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) was used as the anticoagulant for light transmission platelet aggregation (LTA) induced by 20 µM ADP and citrate was used as the anticoagulant for the VerifyNow platelet function studies. The graph below indicates the relationship between the LTA results and the results using the VerifyNow iso-TRAP assay. The endpoint for the Phase 1 study was selected as achieving 80% or more inhibition of LTA because this level of inhibition has been validated as having an antithrombotic effect that results in improved clinical outcomes in patients undergo PCI.(15) The 80% LTA inhibition value in the participants in the Phase 1 study most closely corresponds to 77% inhibition of the VerifyNow iso-TRAP assay, yielding the most true positive (TP) and true negative (TN) values, and the fewest false positive (FP) and false negative (FN) values. As a result, that is the value that we propose to use in this study.

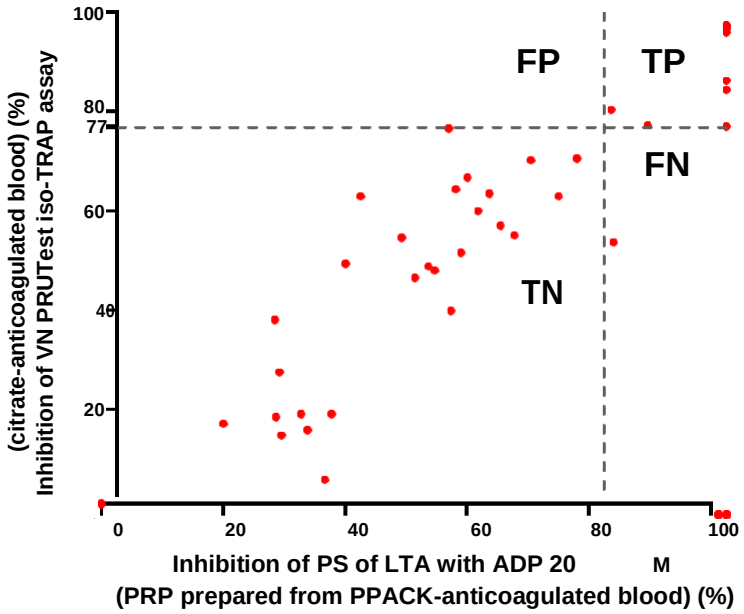


Figure 1. Percent inhibition of platelet function measured by LTA (ADP 20 µM, PPACK-anticoagulated blood) versus VerifyNow PRUTest iso-TRAP assay (citrate-anticoagulated blood). N=11, 50 measurements, of which 39 are post-RUC-4 administration.

As the impact of STEMI on RUC-4 PK and PD in patients is not known, study CEL-02 is designed to assess PD and PK properties of the weight-adjusted dose of RUC-4 (mg/kg) required to achieve 77% or greater inhibition of TRAP-induced platelet aggregation.
In addition, while the BED was reached in the Phase 1 study, the duration of high-grade antiplatelet effect may not be optimal, and there may be benefit in exceeding the BED dose to extend the antiplatelet effect for a longer period of time because oral platelet inhibitors

administered as standard pre-hospital treatment for STEMI patients reach maximum inhibition only after a few hours. The number of patients in each cohort will be evaluated with the objective of achieving 77% or greater inhibition of the VerifyNow iso-TRAP assay after 15 minutes in 7/8 patients, with 7/8 also showing persistence of 50% or greater inhibition of the P2Y12Test BASE assay in citrate at 120 minutes following administration of RUC-4.

In this study, the initial dose of RUC-4 will be 0.075 mg/kg. Following the completion of the first cohort, the decision to escalate to a higher dose level (or lower, or maintain the same dose) will be based on review and analysis of all available safety, tolerability, and PD data by the SRC. The SRC will provide a recommendation to the Sponsor and the Sponsor will inform the PI of the recommendation. A minimum of eight evaluable patients per dose cohort will be enrolled, with two optional dose escalation to levels up to 0.15 mg/kg. The details regarding SRC meeting frequency, data to be reviewed, and the data review process will be included in the SRC charter (see separate document: Safety Review Charter).

1.2.3 Benefit/risk assessment

This is the first study investigating subcutaneous administration of RUC-4 in STEMI patients. Based on the preliminary results of the Phase 1 trial, outlined in Section 1.1 “Clinical experience with RUC-4”, it is anticipated that RUC-4 administration provides an acceptable benefit/risk in this population.

2. OBJECTIVES

2.1 Primary objective

The main objective is threefold:

- To assess the PD properties of a single subcutaneous injection of RUC-4 in STEMI patients presenting to the CCL with planned primary coronary angioplasty
- To assess the PK properties of a single subcutaneous injection of RUC-4 in STEMI patients presenting to the CCL with planned primary coronary angioplasty
- To assess safety and tolerability of RUC-4

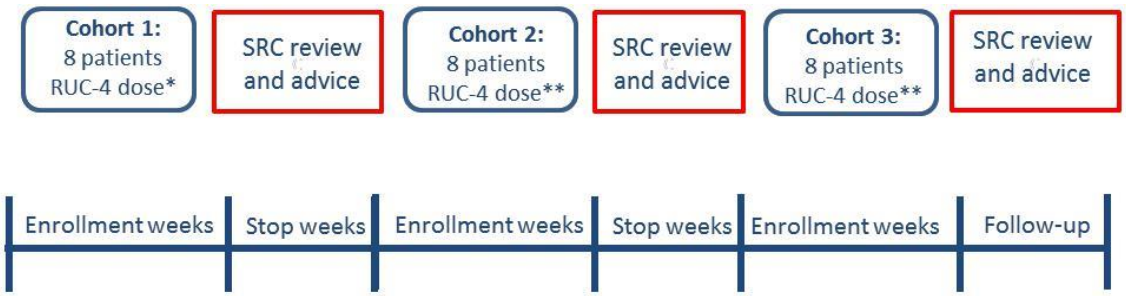
2.2 Secondary objectives

- To assess platelet count at select time points before and after RUC-4 administration
- To assess bleeding events (according to BARC II, III and V criteria for safety assessment and according to ISTH Major and TIMI Major for information only) of a single SC injection of RUC-4 at select time points after RUC-4 administration, discharge and at 15-day and at 30-day follow-up
- To assess intraprocedural thrombosis
- To assess the injection site reactions of a single subcutaneous injection of RUC-4 at select time points after RUC-4 administration and at 15-day and at 30-day follow-up
- To evaluate any differences in PD or PK within each treatment group (gender, weight, BMI, age)

3. STUDY DESIGN

This is an open-label single center Phase 2 study. The anticipated study duration is 19-21 weeks including 1-month follow-up from last patient in; with 14-16 weeks enrollment period including up to two interim analyses for review of data by the SRC at the completion of each dose cohort before a dose-escalation (see Figure 2). The interim analyses will include the day 15 follow up data.

The estimated number of patients to be enrolled is 30, allocated to either of three groups (dose 1 [0.075 mg/kg], dose 2 [0.090 mg/kg], dose 3 [TBD]) to have at least eight patients per group with confirmed acute myocardial infarction with evaluable data.



* Initial dose is 0.075 mg/kg
** Optional dose modifications, with potential elevations up to 0.15 mg/kg. Lowering dose is a potential dose modification.

Figure 2. Study design scheme

The decision to escalate to a higher (or lower, or maintain the same) dose level will be based on review of the interim analysis by the SRC (see Figure 3).

* Initial dose is 0.075 mg/kg
** Optional dose escalation to levels of up to 0.15 mg/kg

Figure 3. Dose escalation scheme

The duration of participation for each patient will be 1 month (± 7 days) including enrollment into study, dosing and follow-up at 15 and 30 days post RUC-4 administration.

4. STUDY POPULATION

4.1 Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria:

1. Patients with STEMI, presenting with persistent chest pain (>30 min) and≥1 mm ST-segment elevation in two adjacent electrocardiograph leads, with >6 mm cumulative ST-segment deviation, in whom the total duration of symptoms to first intracoronary device deployment (excluding a wire) is anticipated to be within 6 hours
2. Adult males and females 18 years of age or older
3. Females must be non-pregnant, non-lactating, and of non-childbearing potential (postmenopausal or surgically sterilized) by history and review of medical record
4. Weight (by history) of between 52 and 120 kg
5. Written informed consent (following short-form of the informed consent form at CCL)

4.2 Exclusion criteria

A potential patient who meets any of the following criteria will be excluded from participation in this study:

1. High probability in the opinion of the cardiologist that current STEMI is caused by stent thrombosis and the previous PCI related to this stent thrombosis is <1 month
2. High suspicion of type II MI
3. Current active coronavirus disease 2019 (COVID-19) infection (criteria according to local guidelines).
4. Out of hospital cardiac arrest (OHCA)
5. Therapy resistant cardiogenic shock (systolic blood pressure ≤80 mm Hg for >30 minutes)
6. Persistent severe hypertension (systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg)
7. Known severe liver disease
8. Known history of severe renal dysfunction (glomerular filtration rate <30 mL/min or serum creatinine >200 mmol/L [>2.5 mg/dL])
9. Known left bundle branch block
10. Requirement of oral anticoagulation (Vitamin K antagonists {VKA} or direct oral antagonists {DOACs})
11. Current treatment with αIIbβ3 receptor antagonist (other than RUC-4)

12. Coagulation abnormality, known bleeding disorder, or history of documented prior hemorrhagic or thrombotic stroke within the past 6 months
13. History of upper or lower GI bleeding within the past 6 months
14. Known clinically important anemia
15. Known clinically important thrombocytopenia (platelet count of less than 150,000/µL)
16. Known history of allergy to any of the ingredients in the RUC-4 formulation (i.e., acetate buffer, sucrose)
17. Major surgery within the past 6 months
18. Life expectancy of less than 6 months
19. Any clinically significant abnormality identified prior to enrollment that in the judgment of the Investigator would preclude safe completion of the study
20. Unwillingness or inability to comply with the requirements of this protocol including the presence of any condition (physical, mental, or social) that is likely to affect the patient's ability to comply with the study protocol

4.3 Sample size calculation

The estimated number of patients to be enrolled is 30, allocated in up to three cohorts of eight evaluable subjects (n=24) at an initial starting dose of 0.075 mg/kg, and anticipating potential drop out of evaluable patients. Each cohort will consist of eight evaluable patients. If a patient is not evaluable, but has been enrolled (e.g. baseline PD and/or PK assessments cannot be performed or the patient prematurely discontinues for whatever reason before the study procedures are performed), extra patients may be enrolled. Based on Phase 1 data, this sample size should be sufficient for an assessment of PD and PK properties of RUC-4 in STEMI patients.

5. TREATMENT OF PATIENTS

All patients will receive a single SC dose of RUC-4 at an initial dose cohort of 0.075 mg/kg in the CCL before coronary angiography/PCI. Dose escalation (or lower dose, or maintain the same dose level) as defined in the SRC (see separate document) can be performed twice with optional dose escalation to levels of up to 0.15 mg/kg.

5.1 Investigational product/treatment

Single-use vials containing RUC-4 and sucrose lyophilized from an acetate buffer will be provided to the site. The RUC-4 staff personnel will prepare the RUC-4 solution for injection based on the patient's weight by reconstituting the lyophilized RUC-4 and sucrose in sterile water for injection. Specific instructions for reconstitution, storage and preparation of the solution for injection and individual patient forms for RUC-4 weight-based dose calculation are provided in a separate Pharmacy Manual.

Table 1. Characteristics product/treatment

Product Name:	RUC-4
Dosage Form:	Solution for injection
Unit Dose:	110 mg RUC-4 per vial (to be reconstituted with 4.8 sterile water for injection)
Route of Administration:	Subcutaneous
Physical Description:	Yellow solution
Manufacturer:	University of Iowa Pharmaceuticals (UIP)

After reconstitution, RUC-4 solutions must be used as follows or discarded. Reconstituted RUC-4 solution in vials and syringes should be stored at 2 to 8 °C (refrigerated) for no more than 24-hours prior to administration. Reconstituted RUC-4 solution in vials and syringes should be stored for no more than 4 hours at room temperature prior to administration.

5.2 Use of concomitant medication

The use of aspirin or an oral P2Y12 antagonist before catheterization is allowed, but not mandated. Heparin is also allowed, as these medications are standard of care for STEMI patients. If heparin was administered pre-hospital, an activated clotting time (ACT) measurement should be performed at the CCL. If the ACT is <200 seconds, additional heparin is recommended. The time, date, dose, and route of administration for aspirin,

P2Y12 antagonists and heparin will be documented. It is not permitted to use αIIbβ3 receptor antagonists.

5.3 Treatment assignment

After the provision of verbal witnessed informed consent (followed by written informed consent before hospital discharge), patients will receive a subject number that will identify their study documents. Following enrollment, subjects will be allocated to a given cohort in sequential order. Treatment assignment will be performed in the CCL using a treatment assignment scheme provided by the Sponsor or designee (see separate document: treatment assignment scheme) for each cohort that specifies the RUC-4 dose level.

5.4 Prohibitions and Restrictions

There are no particular prohibitions and restrictions in this study.

5.5 Treatment after End of Study

Regular standard of care is performed from the provision of informed consent through the last study mandated patient visit.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of the investigational product

The chemical name of RUC-4 is 2-amino-N-(5-(5-oxo-7-(piperazin-1-yl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-2-yl)pyridin-3yl)acetamide. Single-use vials containing RUC-4 and sucrose lyophilized from an acetate buffer will be provided to the site.

6.2 Summary of known and potential risks and benefits

For patients with STEMI, early treatment with RUC-4 could be beneficial by inducing patency of the infarct-related vessel before PCI and by minimizing thrombotic occlusions, thus improving both coronary artery and myocardial microvascular blood flow, resulting in a decrease in infarct size and a reduction in complications of STEMI.
The expected AEs with RUC-4 include increased bleeding due to its antiplatelet effect, thrombocytopenia (based on experience with other αIIbβ3 antagonists), and injection site bruising, bleeding, swelling, and discomfort related to the SC route of administration. The potential to improve the therapy of STEMI - including careful selection of patients to eliminate those at greater risk of bleeding or other AEs, careful monitoring of patients, single-dose RUC-4 administration, and the anticipated short time of exposure to antiplatelet effects of RUC-4 balances the risks to patients and makes the benefit-risk ratio favorable. For a more comprehensive overview of the AEs seen with this product in the previous study see the Investigators Brochure.

Due to the envisioned procedure (PCI) and the specified patient population, the following (S)AEs are expected to occur in this study population: cardiac events including death, recurrent MI, urgent target vessel revascularization, dissection, distal embolization, clinical instability, bleeding (dependent on the origin) and stroke.

6.3 Description and justification of route of administration and dosage

RUC-4 solution for injection will be administered by SC injection. In brief, single-use syringes and needles will be used, and the injection will be given at a 90-degree angle to a pinched region of the skin on the outer aspect of the upper arm or, if neither upper arm is a suitable site, the lateral thigh. Selection of the injection site will be based on clinical assessment. The selected injection site should be free of skin lesions, scars, tattoos, and bony prominences; should not be near the site used for blood draws; should not interfere with obtaining blood pressure or ECGs; and should be readily visible for monitoring of the injection site. A sterile

gauze pad should be applied to the injection site and gentle pressure should be applied for 2 minutes (timed) after the injection. If there is bleeding at the injection site when the pressure is release, the pressure should be re-applied for an additional 2 minutes (timed). The time and date of administration and anatomic location of the SC injection of study drug will be recorded on the electronic case report form (eCRF).

6.4 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling of the investigational medicinal products will be done according to the relevant Good Manufacturing Practice (GMP) guidelines.

6.5 Drug accountability

Study drug accountability throughout the study must be documented and inventory and accountability records must be kept by the PI/pharmacist. The following guidelines relating to study drug accountability will be applied:

- The PI agrees not to supply study drug to any persons except the patients in this study.
- The PI/pharmacist will keep the study drug at the CCL at a secure storage facility under controlled storage conditions as required by the study drug label, accessible only to those authorized by the PI to dispense the study drug.
- The pharmacist will maintain a study drug inventory. The inventory will include details of material received and a clear record of when study drug was dispensed, how much was dispensed, and to which patient it was dispensed.
- The PI/pharmacist agrees to conduct a final drug supply inventory and to record the results of this inventory on the drug accountability record at the conclusion or termination of this study. It must be possible to reconcile delivery records with those of used and returned study drug. Any discrepancies must be accounted for. Appropriate forms of deliveries and returns must be signed by the person responsible.

Used or unused study drug may be collected by Clinical Trial Monitors (CTMs)/Clinical Research Associates (CRAs) or destroyed at the study site according to standard institutional procedures if the Sponsor agrees with the procedure, and after drug accountability has been conducted by the Sponsor or representative, unless otherwise approved. A copy of the standard institutional procedure for destroying investigational drugs must be provided to the Sponsor or designee upon request for review and approval before the first onsite destruction. Unused study drug not destroyed at the site must be returned to the Sponsor designated facility at the end of the study or upon expiration.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Primary study parameters/endpoints

- Inhibition of TRAP-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and 240 minutes after administration of RUC-4 (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3)
- RUC-4 concentration (ng/mL) versus time profiles (at baseline and at 15, 45, 90, 120 and 180 minutes after administration of RUC-4) and associated PK parameters
- Safety and tolerability parameters at baseline and at hospital discharge

7.1.2 Secondary study parameters/endpoints

- Platelet count (µL) at baseline, and at 15, 45, 90, 120 and 180 minutes after administration of RUC-4 and at hospital discharge
- Bleeding events (according to BARC II, III and V criteria for safety assessment and according to ISTH Major and TIMI Major for information only) at baseline, discharge and at 15-day and at 30-day follow-up
- Intraprocedural thrombosis (assessed by PI)
- Injection site reactions at baseline, 1-hour post-PCI, hospital discharge, and at 15-day and at 30-day follow-up
- Inhibition of ADP-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and 240 minutes after administration of RUC-4 (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3)
- Differences in PD or PK among the patients (gender, weight, BMI, age)

7.2 Randomisation and blinding

The study is not randomised or blinded, since no comparator will be used and the primary objectives of the study are independent laboratory measurements of PD and PK. In this study PD and PK data will be compared to PD and PK data of patients enrolled in the Phase 1 study (CEL-01), including the placebo group. The CEL-02 study will assess the effect of RUC-4 in STEMI patients and investigate whether the drug is still effective in sicker patients with potential decreased skin perfusion.

7.3 Study procedures

This study will be conducted in patients with documented STEMI with onset of the cardiac ischemic symptoms within the 6 hours before enrollment and for whom fast revascularization is a major objective for improving prognosis. See Appendix I for an overview of the study procedures.

Screening & enrollment:

Presentation to CCL:

- Patients will be evaluated for study entry in the CCL.
- If the eligibility criteria are met, witnessed verbal IC will be obtained and the patient enrolled in the study will be assigned to treatment cohort.
- The diagnostic ECG (ambulance ECG) will be used for evaluation of STEMI (inclusion criterion #1).
- After witnessed verbal IC, the patient may be enrolled into the study.
- Subsequently recording of demographics, concomitant medications including use of relevant antiplatelet and anticoagulation medications within 7 days, relevant medical history, vital signs, weight & height (by history) and alcohol use will be performed.

Pre-study drug administration (Baseline):

- Two blood samples for platelet aggregation measurement by VerifyNow will be obtained.
- Blood samples for PK analysis will be obtained.
- In addition to the labs obtained for standard of care, at baseline, the following blood samples will be collected: Hb1Ac, hemoglobin, creatinine, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (see investigators brochure Section 4.2.5.3), platelet count, NT-ProBNP, CK, CK-MB and high sensitivity troponin T, ACT, and fibrinogen.
- Adverse events (AEs) will be collected beginning after witnessed verbal IC is obtained.
- Assessment and inspection of the planned injection site will be done (see Appendix 3).

Test dose:

Pre-PCI:

- The study drug will be administered.
- AEs will be collected.

Angiography & PCI:

- Two blood samples for platelet aggregation measurement by VerifyNow will be obtained at 15, 45, 60, 90, 120, 180 and 240 minutes post RUC-4 administration (n=7) (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3). If needed, additional sample(s) may be obtained for VerifyNow assessment until return toward Baseline is observed.
- Blood samples for PK analysis will be obtained at 15, 45, 90, 120 and 180 minutes post RUC-4 administration (n=5).
- ACT will be repeated after heparin administration. AEs will be collected.
- AEs will be collected.

The following procedure data will be recorded at least (all times based upon the recorded time supplied clock on the clipboard): angiography start time, sheath insertion site location, PCI start time, time of balloon inflation, type and number of stents implanted, PCI end time, puncture site device and puncture site hematoma.

1-hour Post-PCI:

- A 12-lead ECG will be obtained 1-hour (±30 minutes) post-PCI (withdrawal of the wire at the completion of PCI)
- Assessment and inspection of the injection site will be performed. Any injection site reactions will be graded as described in the injection site score appendix (see Appendix 3).
- In addition to the labs obtained for standard of care, the following blood samples will be collected: hemoglobin, creatinine, platelet count, NT-ProBNP, CK, CK-MB and high sensitive troponin T.
- AEs will be collected.

Hospital discharge:

- Confirm written IC has been provided prior to hospital discharge.
- A 12-Lead ECG will be obtained.
- The injection site will be evaluated (see Appendix 3).
- Patients weight and height will be obtained as early as possible after PCI and before discharge
- In addition to the labs obtained for standard of care, the following blood samples will be collected: hemoglobin, creatinine, platelet count, CK, CK-MB and high sensitivity troponin Tat 24-hours post RUC-4 administration.

- In addition to the labs obtained for standard of care, the following blood samples will be collected: hemoglobin, creatinine, ALT, GGT, alkaline phosphatase, platelet count, CK, CK-MB and high sensitivity troponin T at 72-hours post RUC-4 administration or hospital discharge (whichever occurs first).
- AEs will be collected.

Follow-up:

A follow-up phone call to elicit and record AEs, bleeding events, injection site reactions (see Appendix 3), and concomitant medications will be conducted at 15 days (±2 days) and at 30-days (±7 days) after RUC-4 administration.

7.4 PD assessment

Whole blood samples for the VerifyNow™ test should be collected into the provided tubes (Greiner Bio One 2 mL blue-top tubes containing 3.2% sodium citrate) using the standardized protocol outlined in the VerifyNow Laboratory Reference Manual for VerifyNow™, using minimum tourniquet pressure. All specimens must equilibrate at room temperature (20-25°C) for a minimum of 10 minutes after collection before testing, but no longer than 4 hours. Do not place the sample in a water bath or on a rocker plate. Backup samples will be collected at each VerifyNow timepoint. If a result is obtained using the primary sample, the backup sample will be discarded. For complete instructions see VerifyNow Laboratory Reference Manual.

7.5 PK assessment

Blood samples for PK analysis will be collected via direct venipuncture. Detailed instructions on collection, processing, storage, and shipping of the PK blood samples are provided in the Laboratory Reference Manual.

7.6 Withdrawal of individual patients

Each enrolled patient shall remain in the study until completion of the required follow-up period. However, a patient's participation in any clinical study is voluntary and the patient has the right to withdraw at any time without penalty or loss of benefit. Conceivable reasons for discontinuation may include, but are not limited to, the following:

- Patient withdrawal: Patient participation in a clinical trial is voluntary and the patient may discontinue participation (refuse study procedures) at any time without loss of benefits or penalty. The informed consent form will be prepared in such a fashion that a

patient will understand and acknowledge that they can discontinue study procedures, but that they can expect to be followed up by phone, their family members may be contacted, their physician may be contacted or a country death registry may be interrogated as permitted by law to ascertain their clinical endpoint and vital status. After patient withdrawal, only information until withdrawal can be shared with the sponsor, with the exception of any SAEs that should be reported (and associated information will be provided as required to the sponsor).

- Investigator termination: Investigator may terminate the patient's participation without regard to the patient's consent if the investigator believes it is medically necessary. Terminating the participation in the study due to medical reason does not end the need for gathering medical resource use data.
- Lost-to-follow-up: Patient who does not complete the follow-up but has not 'officially' withdrawn from the study (does not apply to missed visits where the patient misses one or more of the follow-up contact points but completes a subsequent one). In order to consider a patient lost-to-follow-up, site personnel should make all reasonable efforts to locate and communicate with the patient at each contact time point. A patient locator service and death registries may be utilized as permitted by local law.
- If the patient misses the consecutive scheduled contact time point and the above-mentioned attempts at communicating with the patient are unsuccessful, the patient will be considered lost-to-follow-up.

Any changes and reason for discontinuation or temporary cessation must be documented on the e-CRF.

7.7 Replacement of individual patients after withdrawal

Lost to follow-up and withdrawn patients will not be replaced.

7.8 End of Study definition

Regular standard of care will be performed from the time of consent through last study visit. The end of study for a given subject is reached when the 30-day assessment has been completed. The study site is considered closed when the close-out visit has been performed. The end of the study as a whole is met when all patients have completed all assessments, all patient data have been cleaned and the study site has been closed.

7.9 Premature termination of the study

A possible reason for early study termination includes that the SRC makes a decision for the early termination of the study per recommendation (Section 8.5).

8. SAFETY REPORTING

8.1 Temporary halt of the study for reasons of patient safety

In accordance to Section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise patient health or safety. The sponsor is responsible for this decision. The sponsor will notify the accredited EC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further confirmatory decision by the accredited EC. The investigator will take care that all patients are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

AEs are defined as any undesirable experience occurring to a patient during the study, whether or not considered related to RUC-4. All AEs reported spontaneously by the patient or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A SAE is any untoward medical occurrence or effect that

- results in death
- is life threatening (at the time of the event)
- requires hospitalisation or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator

An elective hospital admission will not be considered as a SAE.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events including specification on severity and causality assessment. The sponsor will report the SAEs to the accredited EC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported

within a period of maximum 15 days after the sponsor has first knowledge of the serious AEs.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see Section 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product
   - Investigator's Brochure for an unauthorised medicinal product

8.3 Causal relationship

Causality is assessed separately for each individual AE and detailed in the internet-based database (e-CRF). The assessment should be made based on the available information and can be updated as new information becomes available. The assessment is based on whether there is "reasonable causal relationship" to the investigational medicinal product in question:

Table 2. Causal relationship adverse events

Relationship	**Criteria**
Not Related	A clinical event, including laboratory test abnormality, with a temporal relationship to study drug administration that makes a causal relationship improbable, and/or in which other drugs, chemicals, or underlying disease provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the study drug, but that could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the study drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and that follows a clinically reasonable response on re administration (rechallenge) or withdrawal (dechallenge).
Definitely Related	Causal relationship is certain. The temporal relationship between study drug exposure and the AE onset/course is reasonable, there is a clinically compatible response to dechallenge, other causes have been eliminated, and the event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

The PI and Medical Monitor will assess the relationship of an AE to study drug according to the criteria in Table 2 and document the relationship in the patient's clinical record. If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable" the event will be considered to be related to the study drug for the purposes of expedited regulatory reporting.

As the Medical Monitor has overview of all the safety data, the Medical Monitor may have a different opinion to the PI. The Medical Monitor will be able to state this additionally for SAE's in the appraisal of a SUSAR.

In the case of a difference of opinion on causality, both assessments are recorded and the worst case assessment is used for reporting purposes.

8.4 Severity assessment

The severity of each AE will be determined by the Investigator according to the following definitions:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL
Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4: Life-threatening; consequences; urgent intervention indicated.
Grade 5: Death; Death related to adverse event.

8.5 Report AEs, SAEs and SUSARs to sponsor

The events reported spontaneously by the patient or observed by the investigator or his staff will be recorded in the eDREAM system of Diagram B.V. The information to be included is at minimum as follows:

- Study number CEL-02
- Site name and number
- PI name
- Subject identification number, sex, and age
- Details of study drug administration
- The date of the report
- A description of the SAE (event term, criterion for seriousness of the event)
- Causal relationship to study drug
- Severity assessment

Informing the sponsor regarding an event via fax should only be performed, when there is no internet access via the following fax line: 0384262990.
In order to contact the Medical Monitor, please send a message to RUC-4.trial@diagram-zwolle.nl.

8.6 Development Safety Update Report (DSUR)

- DSUR should be prepared after the first authorization of a clinical trial worldwide = Development International Birth Date (DIBD).
- DSUR should be developed in accordance with Guidance for Industry: E2F Development

Safety Update Report [16].

- The Investigator's Brochure in effect at the start of the reporting period should serve as the reference for safety information.
- The data lock point (DLP) for a DSUR reporting period is the last day (or the last day of the month) before the anniversary of the DIBD (the one-year reporting period).
- The DSUR should be submitted no later than 60 calendar days from the DSUR data DLP.

8.7 Follow-up of AEs

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study according to the Dutch regulations, as defined in the protocol.

8.8 Safety Review Committee (SRC)

At the end of each cohort, the SRC will receive an interim analysis of the safety, laboratory, and PK/PD data. After reviewing the interim analysis, the SRC will provide written recommendation to the PI whether to proceed with the same dose, or escalate to a higher dose or lower the dose to be studied in the next cohort. If after cohort 1 it is determined to continue the entire study at 0.075 mg/kg, no additional review is required by the SRC prior to the end of the study. For further information, see the separate SRC Charter.

9. STATISTICAL ANALYSIS

9.1 Statistical Analysis Plan

The principal features of the statistical analysis of the data are described in this Section. A more technical and detailed elaboration will be written in a separate statistical analysis plan.

9.2 Analysis populations

The Safety population will include all enrolled patients who receive a SC dose of RUC-4. All safety analyses will be performed on the Safety population. PK/PD analyses will be performed on the PK/PD Analysis Set. The PK/PD Analysis Set includes all patients who receive a SC dose of RUC-4 and who have evaluable PK and PD data.

9.3 General Methodology

All statistical summaries will be descriptive in nature. No hypothesis testing is planned for primary and secondary analyses. Additional exploratory analyses may be performed and will be documented in the statistical analysis plan. Any deviation from the planned analyses described in this protocol will also be documented in the statistical analysis plan. Data will be summarized by dose group.

All statistical calculations will be performed using SAS version 9.4, unless otherwise specified. All data that will be presented in the form of listings will be sorted by dose group and patient ID. Tabular summaries will be presented based on the following grouping: cohort 1, cohort 2, cohort 3 and overall (all cohort groups combined). For continuous data, summary statistics will include number of observations (n), the arithmetic mean, arithmetic standard deviation (SD), median, quartile 1 (Q1), quartile 3 (Q3), minimum (Min), and maximum (Max); for log-normal data (e.g., the PK parameters: AUCs and C_{max}) the geometric mean and geometric coefficient of variation expressed as a percentage (CV%) will also be presented. For categorical data, frequency counts and percentages will be presented. No imputations will be performed on missing data. All analyses will be based on observed data only, except for rules described in Section 9.5.

9.4 PD Assessment

- Inhibition of TRAP-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and 240 minutes after administration of RUC-4 (the

240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3)

Additionally, the inhibition of ADP-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and 240 minutes after administration of RUC-4 will assess the effects of ticagrelor (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3).

The proportion of subjects that have more than 77% inhibition iso-TRAP induced platelet aggregation at different time points will be determined. A summary of number and percentage of subjects will be presented. Data will be summarized by dose group.

9.5 PK Assessment

9.5.1 PK Analysis

Where possible, the following PK parameters will be determined from blood concentrations at baseline, 15, 45, 90, 120 and 180 minutes after administration of a single SC injection of RUC-4:

C_{max}	Observed maximum blood concentration, determined directly from the concentration-time data
T_{max}	Time required to reach maximum blood concentration, determined directly from the concentration-time data
AUC(0-t)	Partial area under the concentration-time curve to time (where T may be 15, 180 minutes or other as appropriate) will be calculated using the linear trapezoidal rule for increasing concentrations and the log trapezoidal rule for decreasing concentrations.
AUC(0-last)	Area under the blood concentration-time curve from time zero to time of last quantifiable concentration calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations
AUC(0-∞)	Area under the blood concentration-time curve from time zero extrapolated to infinity AUC = AUC(0-last) + C(last) / lambda_z
T½	Apparent terminal half-life will be calculated as T½ = ln2/
CL	lambda_z Clearance CL = Dose/AUC(0-∞)
Vd	Volume of distribution VD = Dose/lambda_z* AUC(0-∞)

PK analysis will be carried out using actual sampling times. PK parameters will be calculated using non-compartmental methods. Concentrations for PK analyses will be used as supplied by the analytical laboratory. The units of concentration and resulting PK parameters, with amount or concentration in the unit, will be presented as they are received from the analytical laboratory.

Concentration values that are below the level of quantification (BLQ) will be set to zero, with defined exceptions as follows:

- Any embedded BLQ value (BLQ value occurring between two quantifiable concentrations) and BLQ values following the last quantifiable concentration in a profile will be set to missing for the purposes of PK analysis.
- If there are late positive concentration values following two BLQ concentration values in the apparent terminal Phase, these values will be evaluated. If these values are considered to be anomalous, they will be set to missing.
- If an entire concentration-time profile is BLQ, the profile will be excluded from the PK analysis.
- If a pre-dose concentration is missing prior to the administration of RUC-4, these values will be set to zero.

The number and percentage of subjects with BLQ and missing values will be summarized by time point for each cohort.

9.5.2 Calculation of AUC

The minimum requirement for the calculation of AUC will be the inclusion of at least three consecutive blood concentrations above the lower limit of quantification (LLOQ), with at least one of these concentrations following C_{max}.

9.5.3 Anomalous Values

If a value is considered to be anomalous by being inconsistent with the expected PK profile, it may be appropriate to exclude this point from the PK analysis. However, the exclusion of data must have a strong justification and will be documented in the raw data and the Clinical Study Report. Embedded BLQ values may be considered anomalous depending on the route of administration and the characteristics of the drug.

9.5.4 Dose proportionality

If applicable, the PK parameters AUC($_{0\text{-last}}$), AUC($_{0\text{-}\infty}$) and C_{max} will be assessed for dose proportionality. To investigate the dose proportionality of AUCs and C_{max}, a statistical analysis using the power model will be conducted. The power model will have the form:

$Y = a*(dose)b$

where Y is the PK parameter, and a and b are the coefficient and exponent, respectively, of the power equation.

By taking the natural logarithm (ln), the power model can be analyzed using linear regression and has the form:

$\ln(Y) = \ln(a)+b*\ln(dose) + error = \alpha + \beta*\ln(dose) + error$

where α is the intercept, and β is the slope, and ln(dose) is based on the dose size for each subject.

Both the intercept and slope will be fitted as fixed effects. If there is sufficient data, the model may also be fit with the intercept and/or slope as random effects depending on the ability of the model to converge and on estimation of variance-covariance matrix. Estimates of slope and intercept along with their 90% confidence intervals will be reported. A minimum of three values per dose must be available for a given parameter to estimate dose proportionality using the power model. Slope of the regression line (b) around unity indicates dose-proportionality, b near 0 implies the response y is independent of dose. The width of the confidence interval of the estimate describes the uncertainty.
Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots, while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model. If the assumptions are grossly violated then alternative analyses will be performed.

9.6 Safety and tolerability

Safety and tolerability parameters will be assessed at baseline and at hospital discharge. I.e. AEs, bleeding events, injection site reactions, vital signs, ECGs, and laboratory tests.

9.7 Secondary endpoints

- Platelet count (µL) at baseline, and at 15, 45, 90, 120 and 180 minutes after administration of RUC-4 and at hospital discharge
- Bleeding events (according to BARC II, III and V criteria for safety assessment and according to ISTH Major and TIMI Major for information only) at baseline, discharge and at 15-day and at 30-day follow-up

- Intraprocedural thrombosis (assessed by PI)
- Injection site reactions at baseline, 1-hour post-PCI, hospital discharge, and at 15-day and at 30-day follow-up
- Inhibition of ADP-induced platelet aggregation (%) assessed by VerifyNow at baseline, and at 15, 45, 60, 90, 120, 180 and 240 minutes after administration of RUC-4 (the 240 minute timepoint is only applicable if the RUC-4 dose is increased in cohort 2 and/or 3)
- Differences in PD or PK among the patients (gender, weight, BMI, age)

Categorical data will be summarized by number of patients and percentages. Continuous data by means, standard deviations, median and range. Data will be summarized by dose group.

9.8 Adverse Events

All AEs must be graded for severity and relationship to study drug per Chapter 8 of the protocol. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) AE coding system, version 22.1 (September 2019). All AEs, severe AEs, serious AEs, deaths (if any), and events resulting in study discontinuation will be listed. A summary of number and percentage of subjects with any AEs by maximum grade will be produced. The number of AEs will also be demonstrated. AEs will be sorted by preferred term in descending order of total incidence. The summary will use the following algorithms for counting the subject:

- **Preferred term row**: Subjects experiencing the same AE preferred term several times with different grades will only be counted once with the maximum grade.
- **Any event row**: Each subject with at least one adverse event will be counted only once at the maximum grade no matter how many events they have.

In addition, the frequency and percentage of AEs (all grades) will be summarized and displayed in descending order of total incidence by system organ class (SOC) and preferred term. In the SOC row, the number of subjects with multiple events under the same system organ class will be counted once.

A separate summary will be provided for study treatment-related AEs. A study treatment-related AE is defined as an AE for which the investigator classifies the relationship to study treatment as “Yes”. A worst case scenario approach will be taken to handle missing relatedness data, i.e. the summary table will include events with the relationship to study

treatment as ‘Yes’ or missing. The summary table will be displayed in descending order of total incidence by PT only.
All SAEs will be tabulated based on the number and percentage of subjects who experienced the event. The number of SAEs will also be demonstrated. The summary tables will be displayed in descending order of total incidence by preferred term only.

A study treatment-related SAE is defined as an SAE for which the investigator classifies the relationship to study treatment as “Yes”. A worst case scenario approach will be taken to handle missing data, i.e. the summary table will include events with the relationship to study treatment as “Yes”.

A treatment-emergent adverse event (TEAE) is defined as an AE that occurs after administration of the dose of study product or that is already present prior to the dose of study product and becomes more severe post-dose. In this study, AEs will be evaluated from verbal witnessed IC through the last follow-up visit. A medical condition that exists at the time of screening will be recorded as an AE only when it deteriorates at any time after the study dose administration.

All TEAEs will be summarized for all subjects in the safety population by maximum severity, relationship to the study drug (as assessed by the Principal Investigator), and initial onset day and time. Serious TEAEs will be summarized by maximum severity and relationship to the study drug. TEAEs leading to treatment discontinuation will also be summarized by maximum severity and relationship to the study drug. The frequency, including the number of TEAEs, the number of subjects experiencing a TEAE, and the percentage of subjects experiencing a TEAE, will also be summarized and displayed. TEAEs summaries will be presented by treatment, and by SOC and PT.

The number and percent of subjects with safety events of special interest (bleeding events, injection site reactions, and abnormal platelet counts) will be provided by dose group.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

- This study will be conducted in accordance with the Declaration of Helsinki and ICH-GCP
- The protocol and patient informed consent will be approved by the Ethics Committee (EC)
- It is the Investigator's responsibility to ensure that each patient signs and dates the EC approved informed consent after the nature of the study has been fully explained
- Investigator Agreement:
    - The Investigator agrees to conduct the study in accordance with the approved protocol
    - The Investigator is required to sign the Investigator Agreement prior to enrolling patients
    - The Investigator agrees not to deviate from or make changes to the protocol without prior knowledge and approval of the sponsor and the EC except where necessary to eliminate an immediate hazard to clinical study patients. In this event, the sponsor and the EC should be notified as soon as possible
    - The Investigator should document and explain any deviation or change from the approved protocol
    - The Investigator agrees to allow monitoring and auditing of all essential study documents by the sponsor and to allow inspection and on-site audits by the appropriate regulatory authorities
    - The Investigator agrees to assure the proper implementation and conduct of the study including those study-related duties delegated to other qualified individuals
    - The Investigator and his/her staff agree to co-operate with sponsor representatives during monitoring and auditing visits to assist with the review of the study data and resolve any discrepancies
    - The Investigator agrees to demonstrate due diligence in recruitment and screening of eligible patients. The sponsor should be notified of any projected delays that may impact the completion of the study.

10.2 Recruitment and consent

Patients planned to be enrolled in the study will be informed about their participation in this study. A patient can be considered enrolled in the study once his or her clinical condition meets

the enrolment criteria and he or she gives verbal witnessed informed consent, according to article 6 WMO (If the subject concerned is unable to write, the consent can be given orally is not able to sign the informed consent, verbal informed consent can be obtained by at least one witness), followed by written informed consent before hospital discharge. The Investigator is responsible to collect and file the signed informed consent forms, according GCP guidelines. Patients unable to be followed for approximately 72 hours after study drug administration and for telephonic follow-up at 15-days and at 30-days, will not be included in the study.

10.3 Compensation for injury

The sponsor/investigator has liability insurance that is in accordance with article 7 of the WMO.

The sponsor has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research patients through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

All site personnel will be trained on the protocol, Good Clinical Practice (GCP) and e-CRF prior to the initiation of the study. Trained and qualified CTMs/CRAs will monitor the study throughout its duration by means of personal visits to the physician's facilities, telephone contacts to the physician or designee, and/or remotely through the e-CRF. A comprehensive, integrated data management plan, including on-line queries and remote data cleaning will be implemented to insure the integrity of the data. All changes to the database will be tracked by an audit trail. The PI and his delegates solely have access to the personal data encryption.

11.2 Monitoring and Quality Assurance

Investigators Responsibilities

- Obtaining approval from the EC
- Obtaining written IC from patients
- Adequate enrollment of patients
- Performing medical procedures
- Adherence to the study protocol
- Following patients according to the study protocol
- Registration of all the study details in patient hospital dossier and e-CRF

Protocol Compliance

The investigator is responsible for monitoring in compliance with the study protocol. The CRA will perform visits to inspect and audit study files including Principal Investigator and Co-investigator curricula vitae, EC approval documentation, patient informed consent forms, and study correspondence logs. When problems are identified, assistance will be given to the appropriate individuals to ensure consistency of data collection procedures and transmission of data forms to the Investigator. The investigator will verify that the data recorded on the procedure forms are correct through review of reports and medical records. All events will be 100% monitored according to the monitoring plan. The additional entries will be monitored via the e-CRF using a domain-alert. Only initials and unique patient numbers in the e-CRF will identify patients.

11.3 Amendments

All substantial amendments will be notified to the EC that gave a favourable opinion. Amendments are changes made to the study after a favourable opinion by the accredited EC has been given. All amendments will be notified to the EC that gave a favourable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the EC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial
- the scientific value of the trial
- the conduct or management of the trial
- the quality or safety of any intervention used in the trial

Non-substantial amendments will not be notified to the accredited EC and the competent authority, but will be recorded and filed by the sponsor. Examples of non-substantial amendments are typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited EC once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious AEs/ SAEs, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited EC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the EC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited EC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited EC and the Competent Authority.

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APPENDIX 1. STUDY PROCEDURES

Study Procedures														
	Screening & enrollment		Test-dose											Follow-ups
	Presentation to cardiac catheterization lab	Baseline: Pre-study drug administration	Pre-PCI	Angiography & PCI							1-hour Post-PCI	24-hours post-study drug administration	72-hours post-study drug administration/ Hospital discharge	15 and 30-day phone contact follow-up[15]
			Dose (D)	D-15	D-45	D-60	D-90	D-120	D-180	D-240				
Inclusion/Exclusion[1]	X												X	
Informed Consent[2]	X													
Demographics	X													
Prior/Concomitant Medications[3]	X													
Medical History	X													
Vital Signs	X													
Height, weight	X												X[14]	
Study drug administration			X											
Verify Now Assay[4]		X		X	X	X	X	X	X	X				
PK Sample[5]		X		X	X		X	X	X					
Laboratory testing[6]		X[10-12]									X[10]	x[6]	X[11]	
12-Lead ECG	X[9]										X[13]		X	
Adverse Events[7]		X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of Injection Site[8]		X									X		X	X

D: Dose, ECG: electrocardiogram, PCI: percutaneous coronary intervention, PK: pharmacokinetic

[1]This study will be conducted in patients with documented STEMI with onset of the cardiac ischemic symptoms within the 6 hours before enrollment and for whom fast revascularization is a major objective for improving prognosis.

[2]Witnessed verbal informed consent is required before enrollment, followed by written IC before hospital discharge.

[3] Prior medications taken within 7 days of provision of IC will be recorded. If heparin, aspirin, or a P2Y12 antagonist is administered or taken by the patient prior to arrival in the CCL, the dose and time of administration should be recorded.

[4] Blood samples for platelet aggregation by VerifyNow assay will be obtained pre-dose (baseline), and at 15, 45, 60, 90, 120, 180 minutes and at 240 minutes post RUC-4 administration (n=7 or 8). The 240 minute timepoint is only applicable if the RUC-4 dose is increased (in cohort 2 and/or 3). If needed, additional sample(s) may be obtained for VerifyNow assessment until return toward Baseline is observed.

[5] Blood samples for PK analysis will be obtained pre-dose (baseline), and at 15, 45, 90, 120 and 180 minutes post RUC-4 administration (n=6).

[6] In addition to the labs obtained for standard of care, the following blood samples will be collected: hemoglobin, creatinine, platelet count, CK, CK-MB, high sensitive troponin T will be collected at baseline, 1-hour post-PCI, 24-hours and 72-hours post RUC-4 administration or hospital discharge (whichever occurs first).

[7]Adverse events will be collected at every visit, beginning after verbal witnessed IC is obtained.

[8] The injection site location will be marked after RUC-4 administration and will be assessed by physical examination pre-dose (baseline) after 1-hour post-PCI, upon hospital discharge and at 15-day and at 30-day follow-up.

[9] Collect diagnostic ECG (ambulance ECG).

[10] In addition to the labs obtained for standard of care, NT-ProBNP will be collected at baseline and at 1-hour post-PCI.

[11] In addition to the labs obtained for standard of care blood samples for ALT, GGT and alkaline phosphatase will be collected at baseline and at 72-hours post RUC-4 administration or hospital discharge (whichever occurs first).

[12] In addition to the labs obtained for standard of care at baseline a blood sample for HbA1c, ACT and fibrinogen will be collected. ACT will be repeated after heparin administration.

[13] Window 1-hour post-PCI ECG is ± 30 minutes.

[14] Patients should be weighed and their heights determined before discharge for comparison to the self-reported weight and height obtained on enrollment.

[15] Window for 15-day follow up is ± 2 days and for 30-day follow-up is ± 7 days.

APPENDIX 2. DEFINITIONS

Bleeding	**Bleeding Academic Research Consortium (BARC)** **Type 0**: no evidence of bleeding. **Type 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. Examples include, but are not limited to, bruising, hematoma, nosebleeds, or hemorrhoidal bleeding for which the patient does not seek medical attention. Type 1 bleeding may include episodes that lead to discontinuation of medications by the patient because of bleeding without visiting a healthcare provider. **Type 2**: any clinically overt sign of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that is actionable but does not meet criteria for type 3, type 4 (CABG-related), or type 5 (fatal bleeding) BARC bleeding. The bleeding must require diagnostic studies, hospitalization, or treatment by a healthcare professional. In particular, the bleeding must meet at least one of the following criteria: First, it requires intervention, defined as a healthcare professional–guided medical treatment or percutaneous intervention to stop or treat bleeding, including temporarily or permanently discontinuing a medication or study drug. Examples include, but are not limited to, coiling, compression, use of reversal agents (e.g., vitamin K, protamine), local injections to reduce oozing, or a temporary/permanent cessation of antiplatelet, antithrombin, or fibrinolytic therapy. Second, the bleeding leads to hospitalization or an increased level of care, defined as leading to or prolonging hospitalization or transfer to a hospital unit capable of providing a higher level of care. Or third, the bleeding prompts evaluation, defined as leading to an unscheduled visit to a healthcare professional resulting in diagnostic testing (laboratory or imaging). Examples include, but are not limited to, hematocrit testing, hemoccult testing, endoscopy, colonoscopy, computed tomography scanning, or urinalysis. A visit or phone call to a healthcare professional during which neither testing nor treatment is undertaken does not constitute type 2 bleeding. **Type 3**: clinical, laboratory, and/or imaging evidence of bleeding with specific healthcare provider responses, as listed below: ***Bleeding Academic Research Consortium type 3a bleeding*** Any transfusion with overt bleeding Overt bleeding plus hemoglobin drop ≥3 to <5 g/dL (provided hemoglobin drop is related to bleeding). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL. ***Bleeding Academic Research Consortium type 3b bleeding*** Overt bleeding plus hemoglobin drop ≥5 g/dL (provided hemoglobin drop is related to bleed). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL.

	Cardiac tamponade Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid) Bleeding requiring intravenous vasoactive drugs ***Bleeding Academic Research Consortium type 3c bleeding*** Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation; does include intraspinal); subcategories confirmed by autopsy, imaging, or lumbar puncture Intraocular bleed compromising vision **Type 4**: Coronary Artery Bypass Graft–related bleeding Perioperative intracranial bleeding within 48 hours Reoperation after closure of sternotomy for the purpose of controlling bleeding Transfusion of ≥5 U whole blood or packed red blood cells within a 48-hour period (only allogenic transfusions are considered transfusions for CABG-related bleeds) Chest tube output ≥2 L within a 24-hour period Notes: If a CABG-related bleed is not adjudicated as at least a type 3 severity event, it will be classified as not a bleeding event. If a bleeding event occurs with a clear temporal relationship to CABG (i.e., within a 48-hour time frame) but does not meet type 4 severity criteria, it will be classified as not a bleeding event. **Type 5**: Fatal bleeding Fatal bleeding is bleeding that directly causes death with no other explainable cause. BARC fatal bleeding is categorized as either definite or probable as follows: Probable fatal bleeding (type 5a) is bleeding that is clinically suspicious as the cause of death, but the bleeding is not directly observed and there is no autopsy or confirmatory imaging. Definite fatal bleeding (type 5b) is bleeding that is directly observed (by either clinical specimen [blood, emesis, stool, etc.] or imaging) or confirmed on autopsy. The site of fatal bleeding is specified as intracranial, gastrointestinal, retroperitoneal, pulmonary, pericardial, genitourinary, or other. Bleeding Academic Research Consortium fatal bleeding is meant to capture deaths that are directly due to bleeding with no other cause. The time interval from the bleeding event to the death should be considered with respect to likely causality, but there is no specific time limit proposed. Bleeding that is contributory but not directly causal to death is not classified as fatal bleeding but may be categorized as other forms of bleeding. Bleeding that leads to cessation of antithrombotic or other therapies may be

	contributory but again would not be classified as fatal bleeding. Bleeding associated with trauma or with surgery may be fatal, depending on whether it was determined to be directly causal or not. **ISTH** **Minor Bleeding** - All non-major bleeds will be considered minor bleeds. Minor bleeds will be further divided into those that are clinically relevant and those that are not. - Clinically Relevant Minor Bleed - A clinically relevant minor bleed is an acute or subacute clinically overt bleed that does not meet the criteria for a major bleed but prompts a clinical response, in that it leads to at least one of the following: - A hospital admission for bleeding, or - A physician guided medical or surgical treatment for bleeding, or - A change in antithrombotic therapy (including interruption or discontinuation of study drug). **Major Bleeding** - Fatal bleeding and/or - Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome and/or - Bleeding causing a fall in hemoglobin level of 2 g/dL (1.24 mmol/L) or more, or leading to transfusion of two or more units of whole blood or red cells. **TIMI** **Minimal** - Any overt bleeding event that does not meet the criteria above - Any clinically overt sign of haemorrhage (including imaging) associated with a <3 g/dL decrease in haemoglobin concentration or <9% decrease in haematocrit **Minor** - Clinically overt (including imaging), resulting in hemoglobin drop of 3 to <5 g/dL or ≥10% decrease in haematocrit - No observed blood loss: ≥4 g/dL decrease in the haemoglobin concentration or ≥12% decrease in haematocrit - Any overt sign of hemorrhage that meets one of the following criteria and does not meet criteria for a major or minor bleeding event, as defined above - Requiring intervention (medical practitioner-guided medical or surgical treatment to stop or treat bleeding, including temporarily or permanently discontinuing or changing the dose of a medication or study drug) - Leading to or prolonging hospitalization - Prompting evaluation (leading to an unscheduled visit to a healthcare professional and diagnostic testing, either laboratory or imaging) **Major** - Any intracranial bleeding (excluding microhemorrhages <10 mm evident only on gradient-echo MRI) - Clinically overt signs of hemorrhage associated with a drop in hemoglobin of ≥5 g/dL or a ≥15% absolute decrease in haematocrit - Fatal bleeding (bleeding that directly results in death within 7 d)
MI	**Myocardial infarction** Clinical classification of different types of myocardial infarction:

	Type 1 Spontaneous Myocardial Infarction Spontaneous myocardial infarction related to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD but on occasion non-obstructive or no CAD. **Type 2 Myocardial Infarction secondary to an ischemic imbalance** In instances of myocardial injury with necrosis where a condition other than CAD contributes to an imbalance between myocardial oxygen supply and/or demand e.g. coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy-/brady-arrhythmias, anaemia, respiratory failure, hypotension, and hypertension with or without LVH. **Type 3 Myocardial infarction resulting in death when biomarker values are unavailable** Cardiac death with symptoms suggestive of myocardial ischaemia and presumed ischaemic ECG changes or new LBBB, but death occurring before blood samples could be obtained, before cardiac biomarker could rise, or in rare cases cardiac biomarkers were not collected. **Type 4a Myocardial infarction related to PCI** Percutaneous coronary interventions (PCI) related MI is arbitrarily defined by elevation of cTn value (> 5 X 99th percentile URL) in patients with normal baseline values (< 99th percentile URL) or a rise of cTn values > 20% if the baseline values are elevated and are stable or falling. In addition, either (1) symptoms suggestive of myocardial ischemia or (2) new ischaemic ECG changes or (3) angiographic loss of patency of a major coronary artery or a side branch or persistent slow or no-flow or embolization, or (4) 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 associated with MI when detected by coronary angiography or autopsy in the setting of myocardial ischemia and with rise and/or fall of cardiac biomarker values with at least one value above the 99th percentile URL. **Type 5 Myocardial infarction related to CABG** Myocardial infarction associated with coronary artery bypass grafting (CABG) related MI is arbitrarily defined by elevations of cardiac biomarker value (> 10 X 99th percentile URL) in patients with normal baseline cTn values (< 99th percentile URL). In addition, either (1) new pathological Q waves or new LBBB, or (2) angiographic documented new graft or new native coronary artery occlusion, or (3) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
ST	**Stent Thrombosis** <u>Timing</u> • Acute stent thrombosis: 0 to 24 hours after stent implantation • Subacute stent thrombosis: 24 hours to 30 days after stent implantation • Late stent thrombosis: 30 days to 1 year after stent implantation • Very late stent thrombosis: 1 year after stent implantation <u>Types</u> *Definite stent thrombosis* • ***Angiographic confirmation of stent thrombosis:***

	The presence of a thrombus‡ that originates in the stent or in the segment 5 mm proximal or distal to the stent and presence of at least 1 of the following criteria within a 48-hour time window: o Acute onset of ischemic symptoms at rest o New ischemic ECG changes that suggest acute ischemia o Typical rise and fall in cardiac biomarkers (refer to definition of o spontaneous MI) o Nonocclusive thrombus: Intracoronary thrombus is defined as a (spheric, ovoid, or irregular) noncalcified filling defect or lucency surrounded by contrast material (on 3 sides or within a coronary stenosis) seen in multiple projections, or persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream. o Occlusive thrombus: TIMI 0 or TIMI 1 intrastent or proximal to a stent up to the most adjacent proximal side branch or main branch (if originates from the side branch). • ***Pathological confirmation of stent thrombosis:*** Evidence of recent thrombus within the stent determined at autopsy or via examination of tissue retrieved following thrombectomy. *Probable stent thrombosis* Clinical definition of probable stent thrombosis is considered to have occured after intracoronary stenting in the following cases: • Any unexplained death within the first 30 days, irrespective of the time after the index procedure, any MI that is: o related to documented acute ischemia in the territory of the implanted stent without angiographic confirmation of stent thrombosis, and o in the absence of any other obvious cause *Possible stent thrombosis* Clinical definition of possible stent thrombosis is considered to have occurred with any unexplained death from 30 days after intracoronary stenting until end of trial follow-up.

APPENDIX 3. MODIFIED INJECTION SITE REACTION GRADING SCALE (17)

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity or single use of non-narcotic pain reliever	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization*
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization*
Erythema/Redness **	2.5 - 5 cm	5.1 - 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling ***	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

ER = emergency room

* While subjects are hospitalized, this could also include transfer to an intensive care unit or other facility.

** In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

*** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Since even after subcutaneous bleeding leading to the appearance of a bruise ceases, it is expected that the blood will dissect into the adjacent subcutaneous regions and that it will take several weeks for the resulting ecchymosis to disappear. As a result, resolution of bruising will be judged based on the assessment of when the active bleeding into the subcutaneous space occurred based on clinical assessment of the appearance of a hematoma or other indicators.

Supplementary Appendix 2: Correlation between LTA and VerifyNow iso-TRAP assays

The correlation between inhibition of ADP (20 μM)-induced light transmission platelet aggregometry on blood anticoagulated with PPACK and the VerifyNow iso-TRAP assay on blood collected in citrate was established by correlating the effects over time of administering 0.075 mg/kg RUC-4 to 11 research participants with stable CAD on aspirin (mean age 66, 58% male, mean weight 87 kg) on both assays[18]. This dose produced high-grade inhibition of platelet function within 15 minutes, with return of platelet function toward normal over the next 2 hours. These data were expressed as percentage inhibition of platelet function relative to the pre-treatment value and analysed by Receiver Operating Curve (ROC) analysis with the binary classifier set at 80%. The area under the curve was 0.97 (95% CI 0.9-1.0). The optimal iso-TRAP assay reaction unit cut-off point correlating with 80% inhibition of ADP-induced LTA was ≥77% inhibition (sensitivity = 90.0%, specificity = 100%). (Bentur et al., submitted for publication).