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ORIGINAL RESEARCH

First Human Use of RUC-4: A Nonactivating Second-Generation Small-Molecule Platelet Glycoprotein IIb/IIIa (Integrin αIIbβ3) Inhibitor Designed for Subcutaneous Point-of-Care Treatment of ST-Segment–Elevation Myocardial Infarction

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BACKGROUND: Despite reductions in door-to-balloon times for primary coronary intervention, mortality from ST-segment–elevation myocardial infarction has plateaued. Early pre–primary coronary intervention treatment of ST-segment–elevation myocardial infarction with glycoprotein IIb/IIIa inhibitors improves pre–primary coronary intervention coronary flow, limits infarct size, and improves survival. We report the first human use of a novel glycoprotein IIb/IIIa inhibitor designed for subcutaneous first point-of-care ST-segment–elevation myocardial infarction treatment.

METHODS AND RESULTS: Healthy volunteers and patients with stable coronary artery disease receiving aspirin received escalating doses of RUC-4 or placebo in a sentinel-dose, randomized, blinded fashion. Inhibition of platelet aggregation (IPA) to ADP (20 μmol/L), RUC-4 blood levels, laboratory evaluations, and clinical assessments were made through 24 hours and at 7 days. Doses were increased until reaching the biologically effective dose (the dose producing ≥80% IPA within 15 minutes, with return toward baseline within 4 hours). In healthy volunteers, 15 minutes after subcutaneous injection, mean±SD IPA was 6.9%+7.1% after placebo and 71.8%±15.0% at 0.05 mg/kg (n=6) and 84.7%±16.7% at 0.075 mg/kg (n=6) after RUC-4. IPA diminished over 90 to 120 minutes. In patients with coronary artery disease, 15 minutes after subcutaneous injection of placebo or 0.04 mg/kg (n=2), 0.05 mg/kg (n=6), and 0.075 mg/kg (n=18) of RUC-4, IPA was 14.6%±11.7%, 53.6%±17.0%, 76.9%±10.6%, and 88.9%±12.7%, respectively. RUC-4 blood levels correlated with IPA. Aspirin did not affect IPA or RUC-4 blood levels. Platelet counts were stable and no serious adverse events, bleeding, or injection site reactions were observed.

CONCLUSIONS: RUC-4 provides rapid, high-grade, limited-duration platelet inhibition following subcutaneous administration that appears to be safe and well tolerated.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NTC03844191.

Key Words: GPIIb/IIIa ■ myocardial infarction ■ platelet inhibitor ■ STEMI

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CLINICAL PERSPECTIVE

What Is New?

- A novel small-molecule platelet glycoprotein IIb/IIIa receptor inhibitor (RUC-4) administered subcutaneously provided rapid (<15 minutes), high-grade (>80%) inhibition of platelet aggregation in response to 20 μmol/L of ADP that returned toward baseline within 2 hours.
- RUC-4 appears to be safe and well tolerated, with no serious adverse events, bleeding, thrombocytopenia, or injection site reactions.

What Are the Clinical Implications?

- Our findings help define a dose(s) of RUC-4 for use in a planned phase 2 trial in patients with ST-segment–elevation myocardial infarction.
- RUC-4 has the potential to improve infarct vessel reperfusion and clinical outcomes if administered at the point of first contact before primary coronary intervention.

Nonstandard Abbreviations and Acronyms

BED	biologically effective dose
BMI	body mass index
CAD	coronary artery disease
GPIIb/IIIa	glycoprotein IIb/IIIa
HV	healthy volunteer
IPA	inhibition of platelet aggregation
PPACK	D-Phe-Pro-Arg chloromethyl ketone dihydrochloride anticoagulant
PS	primary slope
SAE	serious adverse event
STEMI	ST-segment–elevation myocardial infarction

Rapid restoration of normal coronary blood flow in an occluded coronary artery by mechanical recanalization, thrombolytic agent, or a platelet glycoprotein IIb/IIIa (GPIIb/IIIa; integrin αIIbβ3) receptor inhibitor limits the extent of myocardial necrosis and reduces mortality in patients presenting with ST-segment–elevation myocardial infarction (STEMI).[1–4] Primary percutaneous coronary intervention with stent deployment is currently the preferred reperfusion modality for STEMI.[1,2] Despite national initiatives that have reduced median door-to-balloon times to <60 minutes, mortality from STEMI has plateaued[5,6] and focus has turned toward reducing total ischemic time (time from chest pain onset to coronary recanalization) to further limit infarct size and improve clinical outcomes.[7,8] Multiple studies have reported that early (pre–primary percutaneous coronary intervention) therapy with a GPIIb/IIIa inhibitor can increase preprocedural infarct artery blood flow, speed ST-segment resolution, limit infarct size, and improve survival in STEMI,[3,4,8–11] regardless of the presence or intensity of concurrent P2Y12 receptor inhibition.[12,13] However, currently available platelet GPIIb/IIIa inhibitors require intravenous administration as a bolus and continuous infusion controlled by a pump, making their use in urgent situations and/or ambulance settings difficult. Oral P2Y12 receptor inhibitors are easier to administer but are poorly absorbed during STEMI, particularly among patients administered opioid analgesics, and require hours to achieve their maximal effect, even when the pills are crushed.[14–18] P2Y12 inhibitors are less potent than GPIIb/IIIa inhibitors as they target only 1 of the ADP receptors, whereas GPIIb/IIIa inhibition blocks the final common pathway for platelet aggregation regardless of upstream agonist, including thrombin, which is central to the pathogenesis of platelet thrombus formation in acute coronary syndromes.[15,19–22]

Compared with eptifibatide and tirofiban, RUC-4 is a second-generation small-molecule platelet GPIIb/IIIa inhibitor specifically designed to inhibit fibrinogen binding, platelet aggregation, and platelet thrombus formation without inducing conformational changes in the receptor produced by these earlier drugs,[23] or fibrinogen,[24] that result in the receptor adopting a high-affinity ligand binding state, with exposure of otherwise hidden epitopes on the receptor.[25,26] Thus, RUC-4 locks the receptor into an inactive conformation and does not expose epitopes that are potential targets for preformed or treatment-induced antibodies that may contribute to thrombocytopenia occasionally associated with eptifibatide or tirofiban[27] treatment. At the molecular level, this is accomplished by RUC-4 displacing the Mg^{2+} in the metal ion–dependent adhesion site of the β3 integrin subunit rather than coordinating it with a carboxyl group from the aspartic acid in fibrinogen or the analogous carboxyl groups in eptifibatide or tirofiban.[25,26] The negative charge of the carboxyl triggers the conformational change.[23,28] RUC-4 was also designed to be biologically active following subcutaneous administration and highly soluble so that the anticipated total human dose can be obtained with an injectate volume <1.0 mL.[26] These attributes facilitate autoinjector delivery and make RUC-4 suitable for STEMI first-point-of-contact therapy.

This report details the first human use of RUC-4 in a phase 1, dose-escalation study conducted in both healthy volunteers (HVs) and stable, aspirin-treated patients with coronary artery disease (CAD).

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study End Points/Objectives

The study's primary objective was to assess the safety and tolerability of RUC-4 administered subcutaneously in HVs and patients with stable CAD taking aspirin at escalating doses until a weight-adjusted (mg/kg) biologically effective dose (BED) or maximum tolerated dose was identified. The BED was defined as the dose of RUC-4 leading to ≥80% inhibition of ADP-induced platelet aggregation (20 μmol/L) within 15 minutes of subcutaneous administration, with a return toward baseline values within 4 hours in at least 5 of the 6 participants receiving RUC-4 in each cohort. Key secondary study objectives were to assess the pharmacokinetics and pharmacodynamics of escalating doses of RUC-4 administered subcutaneously in HVs and patients with stable CAD receiving aspirin until a weight-adjusted BED or maximum tolerated dose was reached. To inform safety monitoring, binomial distribution was used to calculate the 95% CI of serious adverse event (SAE) rate and the number of SAEs required to reject the null hypothesis (that the true SAE rate is ≤5%, 3%, or 1%). For example, if 3 events are observed when 30 patients are enrolled, then it rejects H0: SAE ≤1% but fails to reject H0: SAE ≤3% or H0: SAE ≤5% (Table S1 and Figure S1).

Study Population

HVs (n=14) or patients with stable CAD receiving aspirin (n=30) were enrolled following obtaining informed consent. To be eligible to participate, candidates had to be aged 18 to 75 years, weigh 52 to 120 kg, and have a body mass index (BMI) of 18 to 40 kg/m^2. Detailed inclusion and exclusion criteria are listed in Table S2. The study was approved by the institutional review board of The Christ Hospital, Cincinnati, OH.

Study Design

This was a double-blind, placebo-controlled, dose-escalation study (www.clinicaltrials.gov—NCT 03844191) in which 2 sentinel participants were first administered RUC-4 in each cohort, and then, following safety review committee assessment, the dose was either escalated or 5 additional participants were randomized 4:1 to RUC-4 versus placebo (Figure 1). The sample size for this study was based on the proportion of patients who had >80% inhibition of the initial slope of platelet aggregation compared with baseline. The overall RUC-4 BED is the minimum dose at which efficacy (>80% inhibition) is at least 83.3% (5 of 6 patients). Clopper-Pearson 95% CIs for estimated efficacy for all possible outcomes from a sample of 6 patients in the treatment group were estimated. By 1000 Monte Carlo simulations from the binomial distribution, the decision rule for choosing the overall RUC-4 BED, based on this sample size, has 73.6% (95% CI, 71%–76.4%) sensitivity considering the true efficacy in the studied population to be 83.3%. This sensitivity increases to

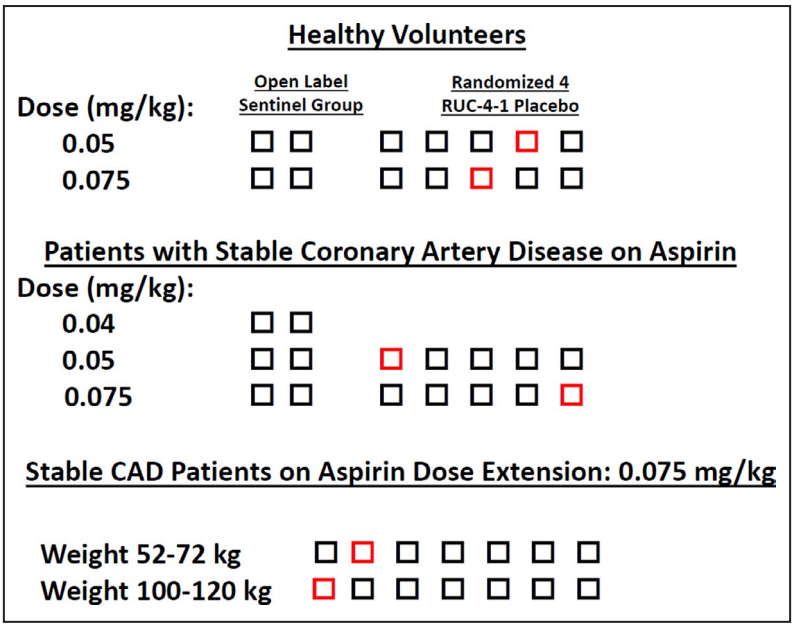

Figure 1. RUC-4 phase I dose-escalation study design.
CAD indicates coronary artery disease; and Red, placebo treated.

88.6% (95% CI, 86.6%–90.5%) if the true efficacy is 90%.

Study Drug Dose and Administration

RUC-4 doses (0.040, 0.050, and 0.075 mg/kg) were administered subcutaneously in the deltoid region using a 25-gauge, 5/8-inch needle attached to a 1-mL syringe with injectate volumes ranging from 0.18 to 0.52 mL (mean, 0.34 mL). An unblinded pharmacist prepared each syringe based on the participant's weight. The pharmacist also prepared placebo syringes, and all syringes were shrouded with brown plastic to mask injectate color so that study personnel remained blinded to treatment. After the study drug was administered, the research nurse applied direct gentle pressure to the injection site for 15 minutes.

Assessment of Platelet Function and RUC-4 Whole Blood Levels

Platelet function and RUC-4 whole blood levels were assessed at baseline (before RUC-4 administration) and at specified time intervals (Table 1) following study drug administration. Inhibition of platelet aggregation (IPA) was measured with light transmission aggregometry (BioData Profiler PAP-8 E) using 20 μmol/L of ADP to activate the platelets. For the platelet aggregation studies, either 4.5 or 9 mL of whole blood were collected into a syringe and immediately dispensed into a 15-mL conical tube containing either 0.5 or 1 mL of 1 mmol/L D-Phe-Pro-Arg chloromethyl ketone dihydrochloride (PPACK) anticoagulant at a 1:10 ratio to have a final total volume of 5 or 10 mL of whole blood containing a final concentration of 100 μmol/L PPACK. PPACK was selected rather than the more commonly employed citrate because citrate chelates Ca^{2+} and Mg^{2+}, reducing their concentrations to below physiological levels, resulting in sensitizing platelets to the effects of RUC-4.[29] Whole blood was centrifuged at 600*g* for 5 minutes at room temperature on day –1 for 10-mL blood samples and 300 g for 5 minutes for subsequent 5-mL samples. In each case, the supernatant platelet-rich plasma was removed and the residual blood was centrifuged at 2000*g* for 9 minutes at room temperature, after which the platelet-poor plasma was removed. Platelet counts were performed on both specimens and when the platelet count in the platelet-rich plasma exceeded 300×10^5/μL, platelet-poor plasma was added to the platelet-rich plasma to adjust the count to 300×10^5/μL. The platelet count exceeded 300×10^5/μL in 30 of the 44 samples tested at the 15-minute time point. IPA was quantified by comparing the primary slope (PS) of the test sample to the PS of platelet aggregation of the baseline sample as previously reported.[30] The PS is a measure of the change in light transmittance per unit time sustained over at least a 15-second period, with data collected every 0.5 seconds, taking into account differences in the lag phase produced by different agonists. The percentage inhibition of the PS relative to the baseline value using the equation (baseline PS–test PS/baseline PS)×100 was selected to avoid the need to choose an arbitrary time point for the comparison to baseline, or using the maximal aggregation, which can occur at different time points. Nonetheless, results using the PS correlated well with results based on maximal aggregation (Figure S2). Platelet-rich plasma was also tested on day –1 with arachidonic acid (1.6 mmol/L final concentration) to assess whether HVs or participants with stable CAD taking aspirin demonstrated an aspirin effect on their platelet aggregation. HVs were to be excluded if the PS of arachidonic acid–induced aggregation was <30% of the PS in response to ADP, and participants with stable CAD taking aspirin were excluded if their PS was ≥15% of the ADP-induced PS. None of the HVs or patients with stable CAD were excluded based on these criteria.

Whole blood RUC-4 levels were assayed by liquid chromatography-mass spectrometry/mass spectrometry on 1-mL samples of whole blood that were immediately added to 4 mL of a mixture of ice-cold acetonitrile:water (30:70 vol/vol). Samples were immediately vortexed and then frozen at –80°C until

Table 1. Time Course of Laboratory Testing and Clinical Evaluations

	Minute											Hour	Hour	Day
Test/Assessment	0	5	15	30	60	90	120	180	240	360	720	24	34	7
Platelet aggregation*		X	X	X	X	X	X	X	X	X		X		
RUC-4 levels†		X	X	X	X	X	X	X	X	X		X		
Platelet Counts		X	X	X	X	X	X	X	X	X		X		X
Clinical evaluations														
Injection site evaluation			X		X			X		X	X	X	X	X

*Percentage reduction of the primary slope of turbidometric platelet aggregation of D-Phe-Pro-Arg chloromethyl ketone dihydrochloride–anticoagulated platelet-rich plasma in response to 20 μmol/L of ADP.

†Whole blood collected into cold acetonitrile:water analyzed by liquid chromatography mass spectroscopy.

analyzed at Charles River Laboratories. Patients who received placebo were excluded from the pharmacokinetic analysis.

Statistical Analysis

IPA levels among HVs and patients with CAD are presented as mean±SD at specified time points following subcutaneous RUC-4 administration. Demographics at baseline are summarized by cohort and presented as median age, mean weight, mean BMI, count of each sex, and count of patients with type 2 diabetes mellitus. Additional exploratory analyses that were not prespecified include a multivariable model to explore whether the weight-adjusted BED outcome differed by covariates of age and sex. To explore the primary efficacy end point as the change in IPA levels from baseline, posttreatment mean IPA levels were compared using a linear mixed effect model suitable for repeated measures. Subject was considered as a random effect, and time, treatment, and treatment-by-time interaction were considered as fixed effects. The model allowed for the intersubject variability of IPA to vary among treatment groups. All exploratory statistical analyses were conducted with SAS version 9.4.6 (SAS Institute Inc).

Pharmacokinetic and Pharmacodynamic Analysis and Modeling

Data from 38 HVs and patients with stable CAD taking aspirin who received RUC-4 and 6 who received placebo were included in the analysis. Sequential population pharmacokinetic and pharmacodynamic analyses were conducted using NONMEM v7.4. Structural pharmacokinetic models that were tested included 1-, 2-, and 3-compartment models with linear elimination processes. Pharmacodynamic models that were tested included direct linear models as well as Emax models. Models were compared using standard model discrimination criteria, including (but not limited to): minimum objective function, quality-of-fit figures, and residual variability. Covariates tested for potential inclusion in pharmacokinetic and pharmacodynamic models included weight, BMI, sex, presence of CAD, or aspirin treatment.

RESULTS

The baseline characteristics and clinical demographics by cohort are shown in Table 2. The majority of participants were men (64% of the HVs and 70% of patients with stable CAD). The median age of the HVs was 45.1 years, while the median age of the patients with stable CAD was 65.0 years. The average BMI among the HVs was 29.0 kg/m^2, compared with 29.5 kg/m^2 for the patients with stable CAD. A total of 2 patients, 6.7% of the patients with stable CAD, had type 2 diabetes mellitus.

Table 2. Demographics and Baseline Characteristics (n=44)

Parameter	HVs (n=14)	Patients With Stable CAD Receiving Aspirin (n=30)
Age, y (minimum, maximum)	45.1 (18, 70)	65.0 (47, 74)
Men/women, n (%)	9 (64.3)/5 (35.7)	21 (70.0)/9 (30.0)
Weight, kg	84.3±14.5	90.0±19.6
BMI, kg/m^2	29.0±4.9	29.5±4.8
Type 2 diabetes mellitus, n (%)	0 (0)	2 (6.7)

Data are mean±SD unless otherwise indicated. BMI indicates body mass index; CAD, coronary artery disease; and HVs, healthy volunteers.

Dose-Escalation and Platelet Function Studies

Ex vivo platelet aggregation by light transmission aggregometry in response to 20 μmol/L of ADP by study drug dose and time following administration of placebo or RUC-4 in doses of 0.050 and 0.075 mg/kg is shown for HVs (Figure 2A) and patients with stable CAD receiving aspirin (Figure 2B). The initial dose of 0.05 mg/kg was selected for HVs based on studies in animals and pharmacokinetic and pharmacodynamic modeling.[26] The next dose of 0.075 mg/kg achieved the BED in 4 of 6 participants, with the other 2 participants having 76.5% and 55.8% IPA at 15 minutes and 68.6% and 73.1% IPA at 30 minutes postdose, respectively, with return toward baseline by 4 hours. Mean IPA at 15 minutes after placebo was 6.9% in HVs. Since the mean IPA was >80% at 15 minutes (mean, 84.7%; median, 88.0%) with return toward baseline by 4 hours, the safety review committee recommended advancing the study to patients with stable CAD taking aspirin. The initial dose administered to patients with stable CAD taking aspirin was reduced (0.04 mg/kg) relative to the starting dose in HVs as a safety measure. Following safety review committee analysis of the first 2 sentinel participants, the RUC-4 dose was increased to 0.05 mg/kg, the same dose used as the initial dose in HVs. Subsequent stable, aspirin-treated patients with CAD were enrolled using the same dose-escalation randomized design format. The 0.075-mg/kg dose in this population achieved the BED in 5 of 6 participants, with a mean 15-minute IPA >80% (mean±SD, 88.9%±12.7%; median, 90.1%) and return toward baseline by 4 hours. Mean IPA at 15 minutes following placebo in the stable CAD population was 14.6%. The 1 participant who did not achieve the BED had 65.4% IPA at 15 minutes and 86.5% IPA at

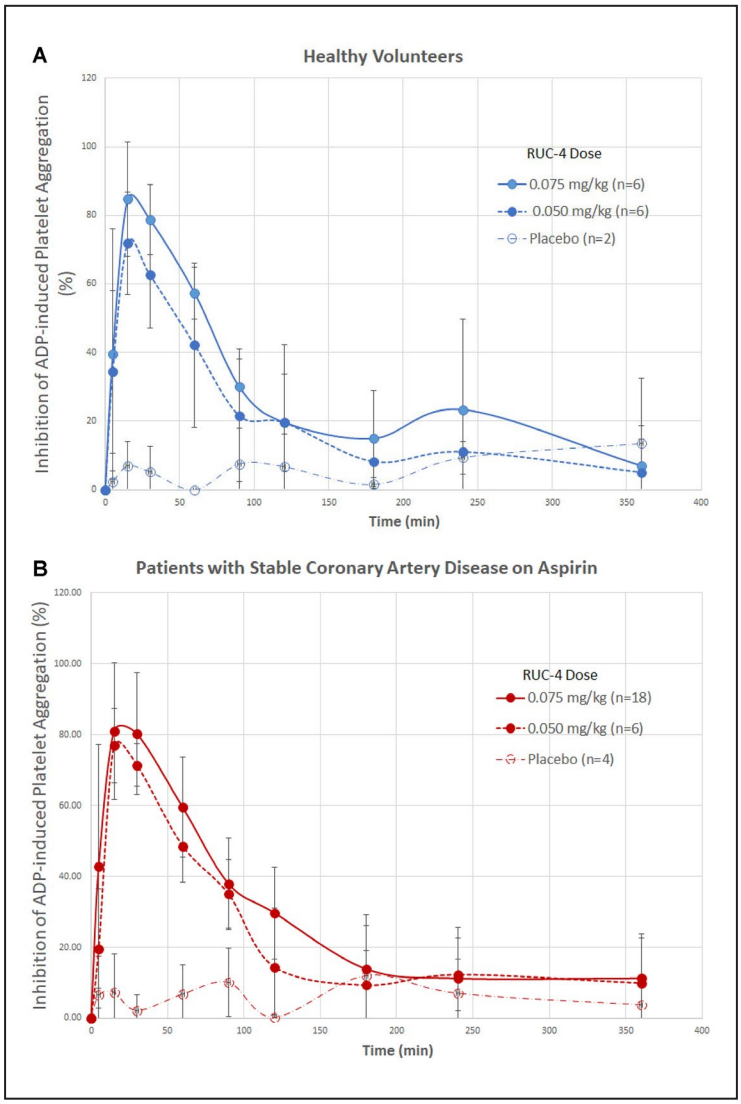

Figure 2. Inhibition of ADP-induced platelet aggregation over time after subcutaneous RUC-4 in (A) healthy volunteers, and (B) patients with stable coronary artery disease receiving aspirin.

30 minutes, with return toward baseline by 4 hours. To assess whether the pharmacokinetics and pharmacodynamics of RUC-4 are affected by weight, enrollment was extended in the population with stable CAD taking aspirin at the same dose (0.075 mg/kg) to relatively low-weight (52–72 kg) and high-weight (100–120 kg) participants. The BED was achieved in 6 of the 12 additional participants (1 of 6 in the low-weight group and 5 of 6 in the high-weight group); the means and median values for the dose-expansion groups were 63.2%±21.4% IPA at 15 minutes and 67.8%±14.7% IPA at 30 minutes in the low-weight group and 88.0%±14.4% at 15 minutes and 85.4%±21.0 % IPA at 30 minutes in the high-weight group. Mean IPA at 15 minutes after placebo in the dose-expansion population was 0.0%. Summary data on the percentage of IPA in patients with stable CAD at baseline and postbaseline are summarized in Table S3. Results from the exploratory multivariable logistic regression indicate that RUC-4 weight-adjusted BED outcomes did not differ by sex or age among patients with stable CAD (Table S4). The exploratory mixed effect model for repeated measures indicated a significant treatment by time interaction (P>0.0001), showing there was a significant change in IPA over time in treatment groups when compared with placebo (results by treatment group are shown in Table S5).

Pharmacokinetic Data

Mean concentration-time profiles of RUC-4 at each dose level are shown in Figure 3 and the relationship between platelet inhibition and RUC-4 blood levels for

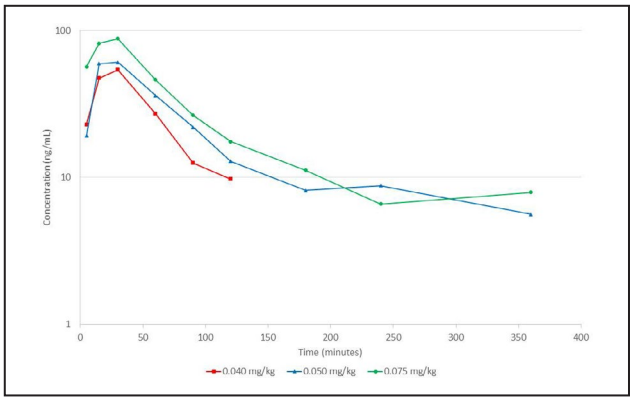

Figure 3. Mean concentration time profiles of RUC-4 by dose level (semi-log scale).

all treated patients is shown in Figure 4. There was a close correlation of RUC-4 blood levels and IPA in the range of ≈20 to 100 ng/mL, corresponding to ≈20% to 100% IPA. Further analysis of the pharmacokinetics/pharmacodynamics by weight, sex, BMI, and aspirin treatment status is shown in Figure 5. Of these variables, only weight significantly affected drug clearance as defined by area under the curve/total dose. Aspirin did not significantly affect RUC-4 pharmacokinetics or pharmacodynamics as judged by the area under the curve, IC50, and clearance values in either cohort (IC50 values 34.7±7.9 with versus 34.8±6.7 ng/mL without aspirin).

Population Pharmacokinetic/Pharmacodynamic Model

The pharmacokinetics of RUC-4 was described by a 2-compartment model with first-order (linear) absorption and elimination processes. A lag time (Tlag) was also included to account for delay before drug absorption. Select pharmacokinetic and pharmacodynamic data derived from the 2-compartment model are shown in Tables S6 and S7. The following individual pharmacokinetic parameter estimates were obtained and expressed as geometric mean (geometric coefficient of variation): absorption rate constant=0.135 min^{-1} (47.9%), Tlag=2.85 minutes (16.8%), apparent central volume of distribution=48.4 L (44.2%), apparent peripheral volume of distribution=221 L (8.5%), apparent total clearance=0.485 L/min (36.7%), and apparent intercompartmental clearance=0.527 L/min (19.2%).

The only statistically significant covariate included in the pharmacokinetic model was weight on clearance and volume of distribution parameters. Covariates such as BMI, sex, presence of CAD, or aspirin treatment did not appear to influence the

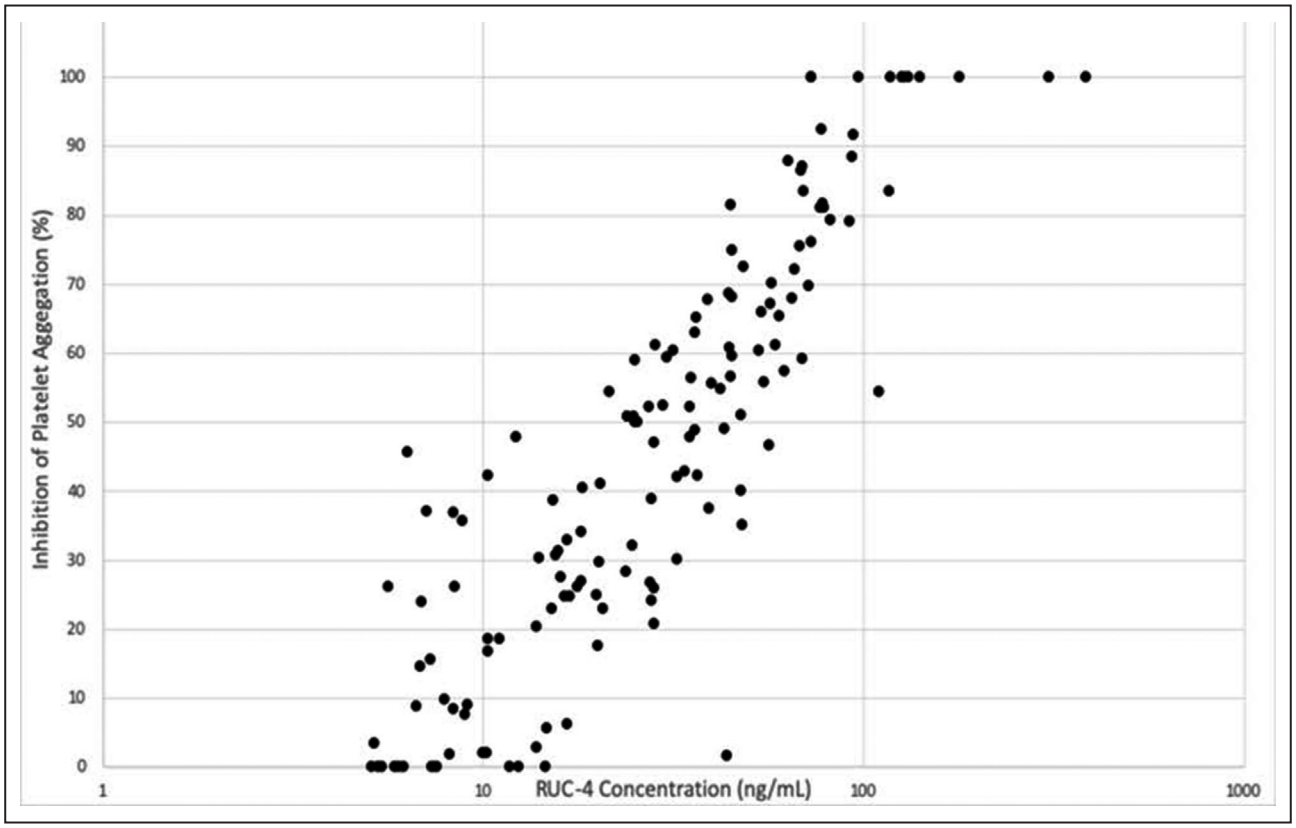

Figure 4. Correlation between inhibition of platelet aggregation and RUC-4 concentration.

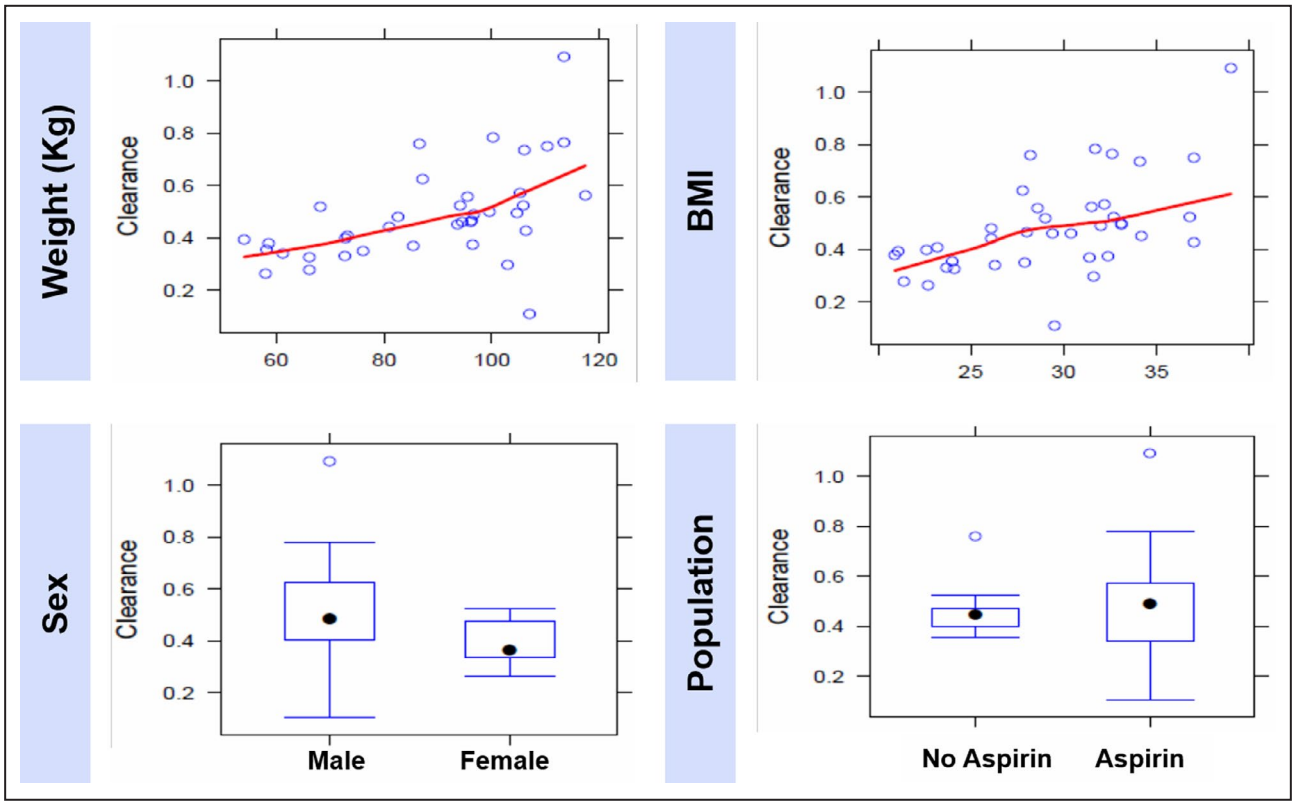

Figure 5. Effect of sex, weight, body mass index (BMI), or aspirin on clearance (area under the curve/ total dose).
Only weight significantly influenced the pharmacokinetic model.

pharmacokinetics of RUC-4. The relationship between clearance and covariates is illustrated in Figure 5. Overall, the model described the data well, with a residual variability of 17.4%. One CAD participant in the dose-expansion group had much higher whole blood levels of RUC-4 than the other participants at the 5, 15, 30, and 60 minutes time points (384, above the upper limit of quantification [500], 307, and 131 ng/mL respectively) compared with group mean values excluding this participant (26.8±26.5, 74.3±38.6, 71.3±30.5, and 40.3±7.3 ng/mL, respectively). These levels correlated with 100% IPA at each of these time points as well as at 60 minutes. Review of RUC-4 formulation, administration, and blood mass spectrometry analysis failed to identify any explanation for this isolated observation. Because this individual was in the higher-weight group, to ensure that the inclusion of this result did not affect the observed weight effect of RUC-4 on clearance, the data were recalculated after excluding this participant's values and the relationship remained significant.

The relationship between RUC-4 concentrations and drug response (defined as IPA) was described by a direct model and a sigmoidal Emax relationship, with maximal percentage inhibition fixed at 100% (Figure 4). The following individual pharmacodynamic parameter estimates were obtained and expressed as geometric mean (geometric coefficient of variation): concentration associated with 50% of maximal effect=33.9 ng/mL (21.9%) and gamma (coefficient)=1.31. No covariates appeared to influence the pharmacodynamics of RUC-4, which suggests that the pharmacodynamic effect of RUC-4 is not influenced by weight, BMI, sex, presence of CAD, or aspirin treatment. The pharmacodynamic model characterized the data well, with a residual variability of ≈31%, considering that pharmacodynamic data are generally more variable than pharmacokinetic data.

As shown by its lack of statistical significance in the pharmacokinetic or pharmacodynamic models, aspirin did not significantly affect RUC-4 pharmacokinetics or pharmacodynamics. Geometric mean (geometric coefficient of variation) clearance values were similar in both cohorts (apparent total clearance 0.484 L/min [43.5%] with aspirin versus 0.487 L/min [17.4%] without aspirin), and IC50 values were also comparable between cohorts (IC50 value 33.8 ng/mL [23.3%] with aspirin versus 34.2 ng/mL [19.5%] without aspirin).

Safety Measures

No SAEs were observed, and the majority of adverse events were graded as mild, with none leading to study

Table 3. Safety Measures in HVs

	RUC-4		
	Placebo (n=2)	0.05 mg/kg (n=6)	0.075 mg/kg (n=6)
No. of TESAEs	0	0	0
Patients reporting at least 1 related TEAE with CTCAE grade ≥3, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting at least 1 bleeding AE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting at least 1 injection site reaction adverse event, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting a non-AE bruising at the injection site event, n (%)	0 (0.0)	2 (33.3)	0 (0.0)
Grade 1, n (%)	0 (0.0)	2 (33.3)	0 (0.0)
Grade 2, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting a TEAE of bruising at the injection site, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

AE indicates adverse event; CTCAE, common terminology criteria for adverse event; HVs, healthy volunteers; TEAE, treatment-emergent adverse event; and TESAE, treatment-emergent serious adverse event.

drug discontinuation (Tables 3 and 4). Bleeding events were uncommon (3 patients), mild (modified Bleeding Academic Research Consortium type 1), and limited to the injection site.

Concurrent aspirin therapy did not appear to influence bleeding. Injection site reactions (including bruising) were mild. There were no drug-related changes in laboratory values and platelet counts were stable through the course of treatment (Tables 3 and 4).

DISCUSSION

The major findings of this first-in-human study of RUC-4 are: (1) prompt onset of action/therapeutic effect, with blood drug levels and platelet inhibition peaking within 15 minutes of subcutaneous administration; (2) potent and predictable therapeutic effect, with high-grade platelet inhibition (mean values of >80% inhibition of aggregation in response to 20 µmol/L of ADP) achieved across the study populations; and (3) tolerability as reflected by the absence of significant adverse events, including no clinically significant bleeding, injection site reactions, or chemistry or hematology laboratory abnormalities. These observations, in addition to the relative ease of subcutaneous administration, make RUC-4 a potentially attractive candidate for STEMI first point-of-care or self-administered therapy. In this context, both the time course and intensity of platelet inhibition without the need for intravenous administration, distinguish RUC-4 from other GPIIb/IIIa platelet–inhibiting therapies. Indeed, as the first platelet function assay was performed on blood obtained 15 minutes following RUC-4 treatment, the time point for maximum platelet inhibition could have occurred even earlier. Importantly, despite the success of national initiatives in reducing

Table 4. Safety Measures in Patients With Stable CAD Receiving Aspirin

	Dose Escalation				Dose Expansion		Total	Total RUC-4
		RUC-4				RUC-4		
	Placebo (n=2)	0.04 mg/kg (n=2)	0.05 mg/kg (n=6)	0.075 mg/kg (n=6)	Placebo (n=2)	0.075 mg/kg (n=12)	Placebo (n=4)	0.075 mg/kg (n=18)
No. of TESAEs	0	0	0	0	0	0	0	0
Patients reporting at least 1 related TEAE with CTCAE grade ≥3, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting at least 1 bleeding AE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting at least 1 injection site reaction AE, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting a non-AE bruising at the injection site event, n (%)	0 (0.0)	0 (0.0)	2 (33.3)	4 (66.7)	0 (0.0)	5 (41.7)	0 (0.0)	9 (50.0)
Grade 1	0 (0.0)	0 (0.0)	2 (33.3)	4 (66.7)	0 (0.0)	5 (41.7)	0 (0.0)	9 (50.0)
Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Patients reporting a TEAE of bruising at the injection site, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

AE indicates adverse event; CAD, coronary artery disease; CTCAE, common terminology criteria for adverse events; TEAE, treatment-emergent adverse event; and TESAE, treatment-emergent serious adverse event.

door-to-balloon treatment time delays, onset of chest pain symptom-to-door (presentation) times remain long and mortality from STEMI has changed little over the past decade.[5,6,31] Efforts at public education and better prehospital/hospital integration into systems for care present an opportunity for reducing total ischemic time by accelerating the prehospital phase of STEMI treatment.[32,33] Pharmacologic interventions (both fibrinolysis and platelet GPIIb/IIIa inhibition) demonstrate greatest benefit if initiated within the first "golden" hour from infarct symptom onset,[34–36] when the occluding coronary thrombus is platelet-rich and dynamic. Although the very early intravenous administration of platelet GPIIb/IIIa inhibitors before primary percutaneous coronary intervention has been demonstrated to facilitate coronary reperfusion, limit infarct size, and improve survival in STEMI,[3,4,8–11] regardless of the concurrent administration of P2Y12 receptor antagonists,[12,13] this approach has been limited by the requirement for intravenous administration, which may be difficult in urgent or ambulance settings. In this context, a therapeutic agent that can be administered subcutaneously and that rapidly achieves a high degree of platelet inhibition with the capacity to disaggregate platelet-rich thrombus could be an attractive addition to current STEMI care.

The primary pharmacodynamic end point (>80% IPA in response to 20 μmol/L of ADP) was chosen as this target was used to establish the dose regimens for the currently approved small-molecule GPIIb/IIIa antagonists,[37–39] and, as reviewed by Jennings et al,[39] this level of platelet inhibition correlates with a reduction in periprocedural primary percutaneous coronary intervention major adverse cardiovascular events, including myocardial infarction and stent thrombosis, as well as clinical benefit in the treatment of acute coronary syndromes.[40–43] Studies with small-molecule GPIIb/IIIa inhibitors have demonstrated that standard citrate anticoagulation, which works through divalent ion chelation, overestimates the platelet-inhibiting effects of these agents,[39] and a similar phenomenon has been observed with RUC-4.[29] To avoid overestimation of IPA, PPACK (which does not chelate divalent cations) was used as an anticoagulant for platelet aggregation studies in this trial.

The apparent safety and tolerability of RUC-4 in the present study is noteworthy. This observation may, in part, be related to the limited time course of potent platelet inhibition. The platelet-inhibiting effects of RUC-4 are designed to resolve within 2 to 3 hours as the effects of the less potent P2Y12 inhibitors become manifest.[14,17] If studies in patients with STEMI indicate the need for a longer duration of high-grade platelet inhibition by RUC-4, this objective may be achieved by increasing the dose or by administering a second dose. Both the current study as well as preclinical studies in nonhuman primates[29] have demonstrated a direct relationship between dose and duration of inhibition.

Although thrombocytopenia (<100 000 platelets per μL) is an infrequent complication of currently available GPIIb/IIIa inhibitors[27] (≈2.5%–6.0% with abciximab, 1.2%–6.8% with eptifibatide, and 1.1%–1.9% with tirofiban),[44] it may be associated with significant consequences. In the present study, platelet counts remained stable during the course of RUC-4 therapy, with only a single participant demonstrating a transient reduction in platelet count to 120 000/μL. The inclusion of only 40 participants who received RUC-4 in this study precludes accurate estimate of the true incidence of thrombocytopenia following RUC-4 with more widespread use. Nevertheless, if RUC-4 proves to be less frequently associated with thrombocytopenia than current agents, that would support the hypothesis that the thrombocytopenia associated with these agents is attributable, at least in part, to the conformational change they produce in the receptor.

Limitations

Although high-grade platelet inhibition by RUC-4 was consistent and predictable among the study population, participants were clinically stable, and greater variability in response might be observed in acute coronary syndromes, particularly STEMI. In addition, although no significant adverse events including bleeding (limited to minor bruising at injection sites) were observed, the potential interaction of RUC-4 with multiple comorbid conditions and/or concurrent medications was limited by protocol. The limited number of participants included in this first human-use experience limits conclusions regarding risks for bleeding or thrombocytopenia, which require definition in larger populations.

CONCLUSIONS

In this first human-use experience with RUC-4, a novel subcutaneously administered platelet GPIIb/IIIa inhibitor, a dose of 0.075 mg/kg provided rapid (<15 minutes), consistent, high-grade (>80%) platelet inhibition that resolved largely within 2 hours following subcutaneous administration to both HVs and patients with stable CAD taking aspirin. RUC-4 appears to be safe and well tolerated with no significant adverse events, bleeding events, or injection site reactions. The results of this study will help to define dose(s) of RUC-4 to be used in a planned phase 2 study involving patients presenting with STEMI.

ARTICLE INFORMATION

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Supplementary Materials

Figures S1-S2
Tables S1–S7

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SUPPLEMENTAL MATERIAL

Figure S1. Comparison of the primary slope (AU/min) and the maximal platelet aggregation values of all samples tested.

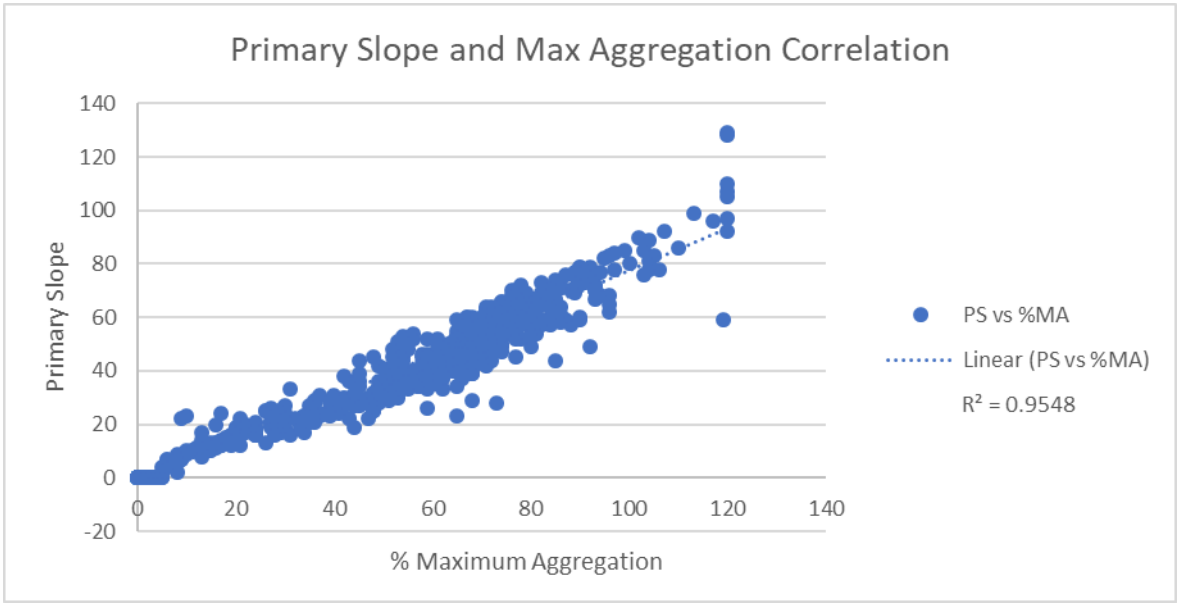

Figure S2. Safety Monitoring: Number of adverse events required to reject H_0 under scenarios of true SAE rate.

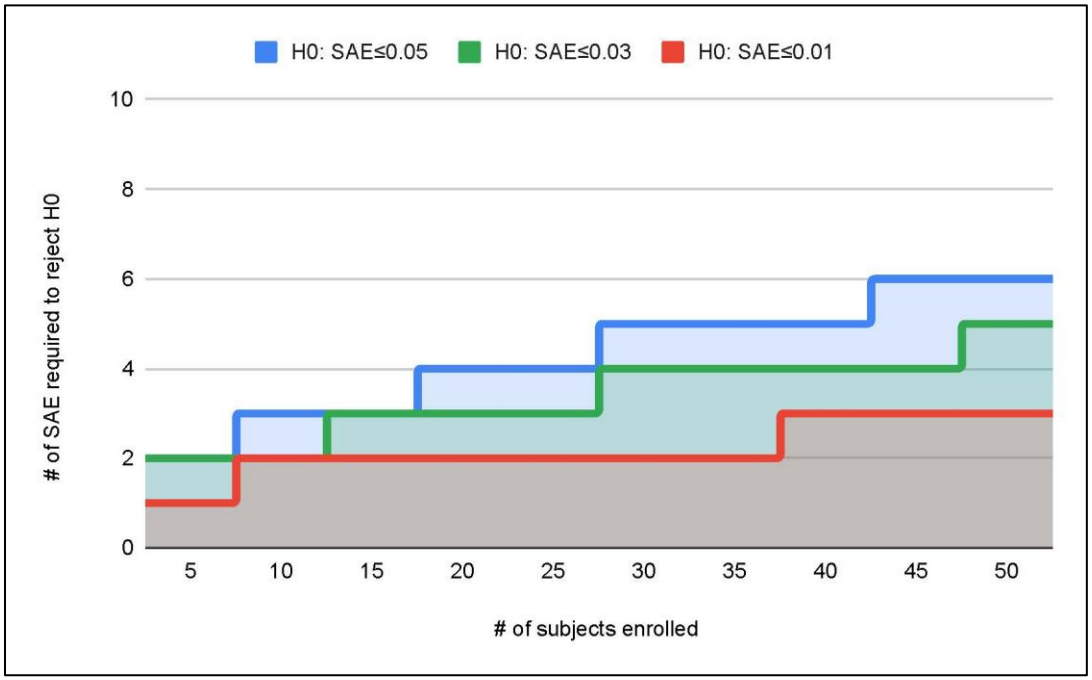

Table S1. Binomial Distribution of Adverse Events to Inform Safety Monitoring.

Number of subjects enrolled	H0: SAE≤ 0.05			H0: SAE≤ 0.03			H0: SAE≤ 0.01		
	# of SAE required to reject H0	95% LCL of SAE rate	P-Value	# of SAE required to reject H0	95% LCL of SAE rate	P-Value	# of SAE required to reject H0	95% LCL of SAE rate	P-Value
5	2	0.0764	0.0226	2	0.0764	0.0085	1	0.0102	0.0490
10	3	0.0873	0.0115	2	0.0368	0.0345	2	0.0368	0.0043
15	3	0.0568	0.0362	3	0.0568	0.0094	2	0.0242	0.0096
20	4	0.0714	0.0159	3	0.0422	0.0210	2	0.0181	0.0169
25	4	0.0566	0.0341	3	0.0335	0.0380	2	0.0144	0.0258
30	5	0.0681	0.0156	4	0.0469	0.0119	2	0.0120	0.0361
35	5	0.0580	0.0290	4	0.0400	0.0202	2	0.0102	0.0479
40	5	0.0506	0.0480	4	0.0349	0.0314	3	0.0208	0.0075
45	6	0.0597	0.0239	4	0.0309	0.0456	3	0.0184	0.0104
50	6	0.0536	0.0378	5	0.0402	0.0168	3	0.0166	0.0138

SAE indicates Serious Adverse Event; LCL, lower control limit.

Table S2. Inclusion and Exclusion Criteria for All Parts, Healthy Subjects Only and Subjects with Stable Coronary Artery Disease on Aspirin Only.

Inclusion	Exclusion
All Parts	
1. Willing and able to give written informed consent.	1. History of prior stroke or clinically significant cardiovascular (e.g., unstable angina, New York Heart Association [NYHA] class II, III, or IV heart failure), dermatologic, endocrine, gastrointestinal (GI), hematologic, infectious, metabolic, neurologic, psychologic, or pulmonary disorder or any other condition, including active cancer that in the opinion of the PI would jeopardize the safety of the subject or impact the validity of the study results.
2. Males and females 18 to 75 years of age, inclusive, at Screening.	2. History of upper or lower GI bleeding requiring intervention or treatment within 12 months of Screening or endoscopic evidence of active peptic ulcer disease within 6 months of Screening.
3. Weight of between 52 and 120 kg, inclusive, and body mass index (BMI) between 18 and 40 kg/m^2, inclusive, at Screening.	3. Bleeding score > 3 on the International Society on Thrombosis and Haemostasis Bleeding Assessment Tool at Screening (See Appendix A).
4. Females must be non-pregnant, non-lactating, and of non-childbearing potential. Females are considered of non-childbearing potential if they are postmenopausal (last menstrual period at least 1 year before Screening and have a serum follicle stimulating hormone [FSH] level greater than 40 mIU/mL) or have been surgically sterilized (documented hysterectomy, tubal ligation, or bilateral oophorectomy) for at least 6 months at Screening. FSH testing is not required for females who	4. Coagulation abnormality, bleeding disorder, or history of documented prior hemorrhagic or thrombotic stroke.

are surgically sterile.	
5. Males must either be surgically sterile for at least 6 months at Screening or agree to use a condom with spermicide from the first dose of study drug through 92 days after the last dose of study drug and must agree not to donate sperm from the first dose of study drug to 92 days after the last dose of study drug.	5. Whole blood donation and/or diagnostic blood evaluation exceeding 500 mL within 8 weeks of Screening.
6. Good general health as determined by no acute illness and no clinically significant abnormal findings on medical history, clinical laboratory test results, vital signs, or physical examination at Screening that in the opinion of the PI would interfere with test product administration, jeopardize the safety of the subject, or impact the validity of the study results; subjects with chronic illness in complete remission are allowed at the discretion of the PI. Out of range laboratory results (e.g., serum alanine aminotransferase [ALT] and aspartate aminotransferase [AST] results that are less than twice the upper limit of normal) may be allowed at the discretion of the PI.	6. Surgical procedure, major injury, or dental procedure with high risk of bleeding within 30 days of Screening.
7. Non-smokers, users of nicotine gum or patches, and smokers who agree to limit their use of tobacco to no more than one pack of cigarettes per day.	7. Supine systolic blood pressure > 150 mmHg or < 90 mmHg or supine diastolic blood pressure > 95 mmHg or < 50 mmHg on 2 consecutive measurements ≥ 10 minutes apart at Screening.
8. Willing to comply with protocol-defined procedures and complete all study visits.	8. A resting heart rate of < 40 beats per minute or > 100 beats per minute when vital signs are measured at Screening.
9. Platelet count of 150,000 /μL to 400,000 /μL and mean platelet volume (MPV) within the normal range.	9. Alcohol consumption of more than 210 mL of alcohol per week, or the equivalent of fourteen 4-ounce glasses of wine or

	fourteen 12-ounce cans/bottles of beer or wine coolers per week within 6 months before Screening.
	10. Marijuana use within the past 3 months, history or presence of substance abuse within the past 12 months, or a positive drug test at Screening. The eligibility of subjects who report using medically-indicated products and/or drugs at Screening that may result in a positive drug test will be evaluated on a case-by-case basis in consultation with the Medical Monitor.
	11. Febrile illness within 14 days of Screening.
	12. Use of metformin for subjects in Part 1 and use of metformin within 24 hours before study drug administration for subjects in Part 2.
	13. Herbal or nutritional supplements/medicines (e.g., St. John's Wort, ginseng, kava, gingko biloba, and melatonin) within 7 days of Screening unless approved by the Medical Monitor.
	14. Participation in another clinical study with an investigational product or device within 30 days of Screening or during the study.
	15. Presence of human immunodeficiency virus (HIV) antibody, hepatitis C virus (HCV) antibody, or hepatitis B surface antigen (HbsAg) in serum at Screening.
	16. Employee of the Sponsor or The Lindner Center staff member directly affiliated with the study, or their immediate family member defined as spouse, parent, child, or sibling.

	Parents, children, or siblings of subjects may not participate in the same dose cohort.
	17. Abnormal platelet aggregation or in vitro inhibition of platelet aggregation pattern by RUC-4 at Check-in Day -1 of the first test dose of study drug.
	18. Receiving or have received in the past 30 days an anticoagulant or fibrinolytic agent.
	19. A cardiac pacemaker.
	20. History of allergy to any of the ingredients in the RUC-4 or placebo formulation (i.e., acetate buffer, sucrose).
Part 1 Only – Healthy Subjects	
10. An interpretable 12-lead ECG without evidence of clinically significant abnormal findings.	21. Medication known to have an impact on platelet function within 30 days of Screening or use of any NSAIDs within 7 days of Screening. Examples include, but are not limited to aspirin, GPIIb/IIIa inhibitors, and P2Y12 inhibitors
	22. Abnormally low response to arachidonic acid-induced platelet aggregation at Check-in Day -1 of each test dose visit.
	23. Screening ECG abnormality that is interpreted by the PI to be clinically significant.
Part 2 Only – Subjects with Stable Coronary Artery Disease on Aspirin	
11. Stable CAD, defined as the following: a. History of documented MI or angina, or evidence of CAD derived from cardiac stress test, or imaging (calcium score [> 100 or > median for	24. Medication known to have an impact on platelet function, with the exception of aspirin, within 30 days of Screening or use of any NSAIDs within 7 days of Screening. Examples include, but are not limited to GPIIb/IIIa inhibitors and P2Y12 inhibitors.

age], angiography, computerized tomography, or magnetic resonance image), and b. Absence of angina, or presence of angina with no change in frequency, duration, precipitating causes or ease of relief for at least 60 days, and c. No ECG or biomarker evidence of myocardial damage in past 60 days.	
12. Blood pressure control achieved with 4 or fewer anti-hypertensive medications, including combination products (e.g., beta blockers, angiotensin-converting enzyme [ACE] inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha-2 receptor agonist, diuretics).	25. More than 4 anti-hypertensive medications required to achieve blood pressure control (See Part 2 inclusion criterion #12).
13. On a stable regimen of aspirin at a dose of 81 to 325 mg/day.	26. Incomplete inhibition of arachidonic acid-induced platelet aggregation at Check-in Day -1 of each test dose visit.
14. In the expansion cohort of Part 2 only, a total of 7 subjects (including at least 2 female subjects) weighing between 52 and 72 kg and a total of 7 subjects (including at least 2 male subjects) weighing between 100 and 120 kg.	27. Acute changes on ECG assessed by the PI as clinically significant.
15. Subjects currently on metformin are required to stop metformin 24 hours before study drug administration (no dose on Day -1) and may resume their metformin no earlier than 8 hours after study drug administration.	

Table S3. Mean and Median IPA by Time point following SC Administration of RUC-4 in Patients with Stable CAD on Aspirin.

		RUC-4 Dose Escalation		
Time Point	**Placebo (N=2)**	**0.04 mg/kg (N=2)**	**0.05 mg/kg (N=6)**	**0.075 mg/kg (N=6)**
Baseline*				
n	2	2	6	6
Mean (SD)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
Median (Q1, Q3)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
5 min post-dose				
n	2	2	6	6
Mean (SD)	11.40 (16.122)	20.50 (28.991)	19.63 (16.825)	43.83 (37.940)
Median (Q1, Q3)	11.40 (0.00, 22.80)	20.50 (0.00, 41.00)	23.80 (0.00, 33.30)	41.35 (6.30, 72.10)
15 min post-dose				
n	2	2	6	6
Mean (SD)	14.55 (11.667)	53.55 (17.041)	76.93 (10.548)	88.92 (12.710)
Median (Q1, Q3)	14.55 (6.30, 22.80)	53.55 (41.50, 65.60)	77.10 (73.00, 86.70)	90.10 (87.90, 100.00)
30 min post-dose				
n	2	2	6	6
Mean (SD)	4.45 (6.293)	44.95 (12.940)	71.47 (6.083)	88.52 (9.207)
Median (Q1, Q3)	4.45 (0.00, 8.90)	44.95 (35.80, 54.10)	72.40 (69.80, 75.00)	85.05 (81.80, 100.00)
60 min post-dose				
n	2	2	6	6
Mean (SD)	8.25 (11.667)	28.65 (5.869)	48.58 (10.185)	63.15 (5.347)
Median (Q1, Q3)	8.25 (0.00, 16.50)	28.65 (24.50, 32.80)	46.20 (42.00, 49.20)	63.25 (59.60, 68.20)
90 min post-dose				
n	2	2	6	5
Mean (SD)	17.90 (5.091)	6.80 (4.243)	35.05 (9.616)	48.94 (7.028)
Median (Q1, Q3)	17.90 (14.30, 21.50)	6.80 (3.80, 9.80)	31.90 (28.60, 43.30)	47.90 (42.90, 52.50)
120 min post-dose				
n	2	2	5	6

Mean (SD)	0.65 (0.919)	1.65 (2.333)	14.48 (16.630)	28.83 (6.899)
Median (Q1, Q3)	0.65 (0.00, 1.30)	1.65 (0.00, 3.30)	5.70 (2.90, 21.70)	27.65 (23.00, 30.80)
180 min post-dose				
n	2	2	6	4
Mean (SD)	10.75 (15.203)	3.00 (4.243)	9.37 (9.651)	2.70 (4.229)
Median (Q1, Q3)	10.75 (0.00, 21.50)	3.00 (0.00, 6.00)	5.80 (1.90, 15.00)	0.95 (0.00, 5.40)
240 min post-dose				
n	2	2	6	6
Mean (SD)	14.10 (8.768)	0.00 (0.000)	12.32 (10.250)	8.00 (11.031)
Median (Q1, Q3)	14.10 (7.90, 20.30)	0.00 (0.00, 0.00)	10.60 (4.80, 21.70)	3.80 (0.00, 12.50)
360 min post-dose				
n	2	2	6	6
Mean (SD)	7.60 (10.748)	0.00 (0.000)	8.43 (12.113)	6.43 (5.042)
Median (Q1, Q3)	7.60 (0.00, 15.20)	0.00 (0.00, 0.00)	3.95 (0.00, 11.70)	9.05 (0.00, 9.80)
24 hours post-dose				
n	2	2	6	6
Mean (SD)	19.30 (13.859)	0.00 (0.000)	9.95 (12.585)	4.08 (4.881)
Median (Q1, Q3)	19.30 (9.50, 29.10)	0.00 (0.00, 0.00)	4.95 (0.00, 18.80)	2.70 (0.00, 7.60)

*Baseline is defined as the last available measurement taken prior to study drug administration of each test dose.
Min indicates minutes; Q1, 25th percentile; Q3, 75th percentile.
Note: Subjects receiving Placebo are pooled together across all cohorts. Subjects that participate in more than one cohort are counted in each cohort.

Table S4. Baseline* Predictors of RUC-4 weight-adjusted BED† reached among patients with stable coronary artery disease on aspirin (N=30).

	Odds Ratio	95% Confidence Interval	P-value
Sex			
Male	(ref)	(ref)	(ref)
Female	0.50	0.097 – 2.578	0.4073
Age	0.99	0.892 – 1.119	0.9846

* Baseline is defined as the last available measurement taken prior to study drug administration of each test dose.

†The BED was defined as RUC-4 leading to ≥80% inhibition of ADP-induced platelet aggregation (20 μM) within 15 minutes of SC administration

Table S5. Mixed Effects Model with Repeated Measures Estimates of Changes in IPA in Patients with Stable CAD on Aspirin.

Treatment Group	Time Point	Difference in IPA Compared to Placebo	95% Confidence Intervals		P-value
0.04 mg/kg n=2	Pre-Dose	(ref)			
	5 minutes post-dose	14.025	-19.5364	47.5864	0.4113
	15 minutes post-dose	46.275	12.7361	79.8139	0.007
	30 minutes post-dose	42.725	9.2294	76.2206	0.0126
	60 minutes post-dose	21.8	-11.6121	55.2121	0.2
	90 minutes post-dose	-3.325	-36.576	29.926	0.844
	120 minutes post-dose	1.325	-31.6143	34.2643	0.9369
	180 minutes post-dose	-9.05	-41.3817	23.2817	0.5819
0.05 mg/kg n=6	Pre-Dose	(ref)			
	5 minutes post-dose	13.1583	-11.8569	38.1736	0.3012
	15 minutes post-dose	69.6583	44.6599	94.6568	<.0001
	30 minutes post-dose	69.2417	44.2756	94.2078	<.0001
	60 minutes post-dose	41.7333	16.8294	66.6372	0.0011
	90 minutes post-dose	24.925	0.1412	49.7088	0.0487
	120 minutes post-dose	13.3964	-11.4484	38.2411	0.2893
	180 minutes post-dose	-2.6833	-26.782	21.4153	0.8266
	240 minutes post-dose	5.2667	-17.9368	28.4701	0.6552
	360 minutes post-dose	4.6333	-16.7447	26.0114	0.6698
	24 hours post-dose	-7.825	-25.1631	9.5131	0.3749
0.075 mg/kg n=17	Pre-Dose	(ref)			
	5 minutes post-dose	36.7299	15.2511	58.2086	0.0009
	15 minutes post-dose	74.106	52.6551	95.5569	<.0001
	30 minutes post-dose	77.9311	56.5182	99.344	<.0001

	60 minutes post-dose	52.4518	31.0921	73.8115	<.0001
	90 minutes post-dose	28.082	6.8249	49.3391	0.0098
	120 minutes post-dose	28.0914	6.9948	49.188	0.0093
	180 minutes post-dose	2.3148	-18.3953	23.0248	0.8259
	240 minutes post-dose	4.1833	-15.6869	24.0536	0.6787
	360 minutes post-dose	7.4667	-10.8404	25.7737	0.4226
	24 hours post-dose	-9.1306	-23.978	5.7169	0.227

IPA indicates inhibition of platelet aggregation.

Subjects are pooled together across all cohorts.

Table S6. Comparison of Individual PK Estimates from Final PK Model With and Without Subject 2019.

	PK Parameters	All Subjects		Excluding Subject 2019	
		Geometric Mean	CV%	Geometric Mean	CV%
Absorption	Tlag (min)	2.84	16.8	2.94	17.4
	Ka (1/min)	0.135	47.9	0.135	52.5
Distribution	Vc/F (L)	48.4	44.2	50.7	32.9
	Vp/F (L)	221	8.53	392	11.0
	Weight exponent on volumes	0.384	N/A	0.493	N/A
Clearance	CL/F (L/min)	0.485	36.7	0.379	38.3
	CLd/F (L/min)	0.527	19.2	0.643	16.9
	Weight exponent on clearances	0.420	N/A	0.755	N/A

CL/F indicates apparent total clearance; CLd/F, apparent intercompartmental clearance; Ka, first order absorption rate constant; N/A, not applicable; Tlag, lag time; Vc/F, apparent central volume of distribution; and Vp/F, apparent peripheral volume of distribution.

Table S7. Comparison of Individual PD Estimates from Final PD Model With and Without Subject 2019.

	All Subjects		Excluding Subject 2019	
PK Parameter	**Geomean**	**CV%**	**Geomean**	**CV%**
C_{50}	33.9	21.9	33.4	24.1
Gamma	1.31	N/A	1.26	N/A