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Double-blind, placebo-controlled Phase II studies of the protease-activated receptor 1 antagonist E5555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease

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Aims Two multicentre, randomized, double-blind, placebo-controlled Phase II studies assessed the safety and efficacy of the oral protease-activated receptor 1 (PAR-1) antagonist E5555 in addition to standard therapy in Japanese patients with acute coronary syndrome (ACS) or high-risk coronary artery disease (CAD).

Methods and results Patients with ACS ($n = 241$) or high-risk CAD ($n = 263$) received E5555 (50, 100, or 200 mg) or placebo once daily for 12 (ACS patients) or 24 weeks (CAD patients). The incidence of TIMI major, minor, and minimal bleeds requiring medical attention was similar in the placebo and combined E5555 (atopaxar) groups (ACS: 6.6% placebo vs. 5.0% E5555; CAD: 1.5% placebo vs. 1.5% E5555). There were no TIMI major bleeds and three CURE major bleeds (two with placebo; one with 100 mg E5555). There was a numerical increase in 'any' TIMI bleeding with the E5555 200 mg dose (ACS: 16.4% placebo vs. 23.0% E5555, $P = 0.398$; CAD: 4.5% placebo vs. 13.2% E5555, $P = 0.081$). The rate of major cardiovascular adverse events in the combined E5555 group was not different from placebo (ACS: 6.6% placebo vs. 5.0% E5555, $P = 0.73$; CAD: 4.5% placebo vs. 1.0% E5555, $P = 0.066$). There was a statistically significant dose-dependent increase in liver function abnormalities and QTcF with E5555. At trough dosing levels in both populations, mean inhibition of platelet aggregation was >90% with 100 and 200 mg E5555, and 20–60% with 50 mg E5555.

Conclusion E5555 (50, 100, and 200 mg) did not increase clinically significant bleeding, although there was a higher rate of any TIMI bleeding with the highest two doses. All doses tested achieved a significant level of platelet inhibition. There was a significant dose-dependent increase in liver function abnormalities and QTcF. Although further study is needed, PAR-1 antagonism may have the potential to be a novel pathway for platelet inhibition to add on to the current standard of care therapy.

Keywords E5555 • Atherothrombosis • Thrombin • PAR-1 • Acute coronary syndrome • Coronary artery disease

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Introduction

Atherothrombosis is the leading cause of death in the industrialized world, and in many countries, including Japan, the incidence of atherothrombosis is increasing as socio-economic changes lead to an increase in coronary risk factors.[1–4] Atherothrombosis involves the sudden rupture of an atherosclerotic plaque within a diseased blood vessel, leading to platelet activation, thrombus formation, and inflammation, which culminate in partial or complete occlusion of blood vessels.[5]

Platelets play a central role in the progression of atherothrombosis[6–9] and have become a key target for therapeutic intervention.[10] Agents that inhibit platelet activation, such as aspirin[11] and $P2Y_{12}$ 5′-adenosine diphosphate (ADP) receptor antagonists,[12] are included in management guidelines for acute coronary syndrome (ACS)[13] and coronary artery disease (CAD).[14] However, these agents do not inhibit the thrombin receptor, which is one of the most potent receptors for platelet activation.[15] Thus, platelets can still be activated and aggregated through thrombin receptor stimulation, even though the $P2Y_{12}$ ADP receptor- and TxA_2-related activation pathways are blocked by the current standard of care treatment.

Thrombin mediates its effects through protease-activated receptor 1 (PAR-1) on the platelet surface. Inhibition of PAR-1 represents a novel approach for reducing platelet activation,[16] which spares other thrombin-mediated effects associated with haemostasis.[17] E5555 (atopaxar) (*Figure 1*) is a low-molecular-weight inhibitor of PAR-1 which has been shown to inhibit platelet aggregation *in vivo* without causing prolongation of bleeding time.[18–20] Other PAR-1 inhibitors revealed antithrombotic activity in an arterio-venous shunt model without lengthening bleeding time.[21] Here, we evaluated the safety and tolerability of oral E5555 in two multicentre, randomized, double-blind, placebo-controlled Phase II studies in Japanese patients with ACS or high-risk CAD.

Methods

Study design and patient population

J-LANCELOT (Japanese-Lesson from Antagonizing the Cellular Effect of Thrombin) studies were two randomized, double-blind, placebo-controlled, parallel-group, Phase II trials which included 12-week treatment for ACS patients (ClinicalTrial.gov identifier: NCT00619164) and 24-week treatment for CAD patients (ClinicalTrial.gov identifier: NCT00540670). Patients were eligible if they were 45–80 years of age.

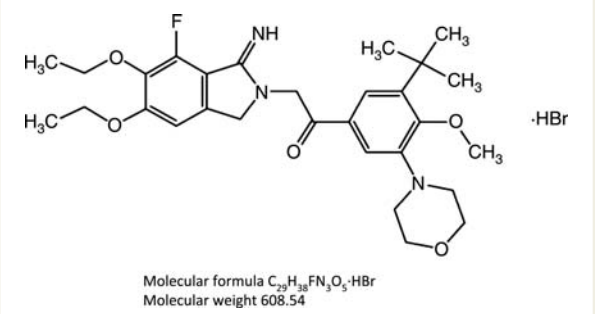

Figure 1 E5555 chemical structure.

For the ACS study, patients were inpatients with non-ST-elevation myocardial infarction (NSTEMI) or unstable angina (UA), with their last symptom occurring within 24 h prior to enrolment in the study. To be eligible for the study, patients needed to have a new or aggravated episode of ischaemic chest pain or have developed any ischaemic symptom at rest or on light activity (such as chest pain lasting for 5 min or longer or requiring sublingual administration of nitrate or a similar treatment). In addition, patients needed to meet one of the following criteria at hospitalization: troponin T, troponin I, or CK-MB >ULN (upper limit of normal) of the institution; ischaemic changes on electrocardiogram (ECG), such as ST depression ≥1 mm (adjacent two leads), inverted T-wave ≥3 mm, or transient elevation of ST not lasting 20 min.

For the CAD study, patients had confirmed CAD defined as one of the following: post-ACS or percutaneous coronary intervention (PCI) (>4 weeks), post-CABG (>12 weeks), angina with documented ischaemia (by ECG or imaging), or angiographically documented stenosis ≥70% of a coronary vessel. Patients also had to be in a high-risk group for CAD, with a history of treatment for diabetes mellitus, a documented history of peripheral artery disease, or a documented history of atherothrombotic transient ischaemic attack (TIA) or stroke for more than 1 year prior to inclusion. All patients had to be receiving aspirin (75–325 mg) for at least 4 weeks before screening.

Major exclusion criteria in both studies were: history of an acquired or congenital bleeding disorder (including coagulopathy or abnormal platelets), history of intracranial bleeding, history of ischaemic cerebral infarction or TIA within the past year or known structural cerebral vascular lesion, evidence of active pathological bleeding at screening or history of bleeding (such as gastrointestinal or genitourinary) from an unknown cause within 24 weeks prior to screening, unstable diabetes mellitus, significant renal impairment defined as serum creatinine >2.0 mg/dL (>176 μmol/L), NYHA class III or IV cardiac failure, documented history of chronic liver disease and/or screening alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3× ULN or total bilirubin >1.5× ULN, oral anticoagulants, or fibrinolytics.

Study protocol

At each site, the study was approved by the Institutional Review Board. All patients included in the study provided written informed consent.

Patients were randomly assigned to four groups (placebo, 50, 100, or 200 mg E5555), received study drug orally once daily for 12 weeks (ACS patients) or 24 weeks (CAD patients), and were followed up for up to 4 weeks after the final dose. In the ACS study, patients received placebo (placebo group) or 400 mg E5555 (all E5555 groups) as a loading dose on Day 1 immediately after randomization.

The primary safety endpoint was the incidence of bleeding events. Bleeding severity was classified according to the CURE (major and minor bleeding)[22] and TIMI (major, minor, and minimal bleeding)[23] definitions (Appendix 1). Bleeding events were adjudicated by the Clinical Evaluation Committee (CEC) which consisted of four blinded members who were not investigators. The secondary endpoint was the incidence of major cardiovascular adverse events (MACEs), such as cardiovascular death, MI, stroke, or recurrent ischaemia. Major cardiovascular adverse events also were adjudicated according to TIMI criteria.

Thrombin receptor-activating peptide (TRAP)-induced platelet aggregation was performed using the turbidimetry method at those study centres able to perform the test. A final concentration of 15 μM TRAP was used for the test. For patients with ACS, platelet

aggregation was measured at baseline, Days 1–4 (during acute phase), and then at Weeks 2, 4, 8, 12, and 16. For patients with CAD, platelet aggregation was measured at baseline (Day 1), and at Weeks 2, 4, 12, 24, 26, and 28. Excluding Day 1 in ACS patients, platelet aggregation was measured at trough level. Platelet aggregation inhibition (PAI) was calculated post-dosing and compared with baseline.

Additional information related to safety, including adverse events, vital signs, 12-lead ECGs, and clinical laboratory tests, was also collected. Blinded 12-lead ECG was conducted by a central ECG analysis institution (Quintiles, India).

Statistical analysis

Efficacy and safety populations included all randomized patients who showed GCP compliance, who received at least one dose of the study drug, and who had at least one post-baseline assessment. Continuous variables are presented as summary statistics. Categorical variables are expressed as frequencies and percentages. Analysis of variance or Fisher's exact test was performed to assess imbalance among groups with respect to baseline demographics and clinical characteristics. Exact Cochran–Armitage's test was performed to assess dose–response relationship with respect to incidence of bleeding events, MACEs, and abnormal hepatic function parameters (ALT and AST). Moreover, comparisons of the incidence between each active group and the placebo group, and all combined active groups and the placebo group, were assessed by relative risk. Each relative risk estimate was accompanied by a two-sided exact *P*-value and exact 95% confidence limits based on the Agresti–Min method. Fisher's exact test was performed to compare the combined active group with the placebo group with respect to the incidence of adverse events and serious adverse events. The Jonckheere test was performed to assess dose–response relationship with respect to QTcF at the last observation carried forward (LOCF) analysis endpoint. The Wilcoxon signed-rank sum test or Wilcoxon test was performed for intra- or inter-group comparisons. Significance levels in the tests were set as follows: two-sided 5% for dose–response relationship and comparisons, and two-sided 15% for imbalance among groups. Statistical analyses were performed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) and StatXact8 (Cytel Inc., Cambridge, MA, USA). The primary objective for both studies was to explore the safety and tolerability of E5555. Therefore, a formal sample size for these studies was not calculated.

All of the data analysis in this paper was conducted by the statistician at Eisai Co., Ltd, and also confirmed by SOC Co., Ltd, in Japan (the statistical representative is Shigeru Tatsuno), a clinical research organization independent from Eisai Co., Ltd. It is these independent analyses that are presented in this paper.

Results

Of 249 patients with ACS and 271 with CAD enrolled in the study, 241 and 263 were randomized to treatment groups, respectively (*Figure 2*). Baseline demographic and clinical characteristics of randomized patients are shown (*Table 1*). Study drug discontinuation rates after randomization in the placebo, 50, 100, and 200 mg E5555 groups were 27.9, 33.3, 32.3, and 32.8% ($P = 0.641$; Cochran–Armitage's test) in ACS patients, and 6.1, 9.5, 12.1, and 25.0% ($P < 0.001$; Cochran–Armitage's test) in CAD patients, respectively. The median date for study drug discontinuation in the placebo group was 12 days, and in the active combined group, it was 14 days in ACS patients.

In patients with ACS, 32.8 and 67.2% of the patients recruited as ACS were UA and NSTEMI patients, respectively. The majority of the patients (95.1% in placebo and 91.7% in E5555 pooled) received both aspirin and thienopyridine as concomitant medications. Of the thienopyridines, 75.5% of the patients were treated with clopidogrel and 24.1% of them were treated by ticlopidine). In patients with CAD, however, less than half of the patients received dual antiplatelet therapy (39.4% in placebo and 42.1% in E5555 pooled) during the study period. In patients with CAD, over 60% reported a previous MI and over 80% reported a previous PCI. Less than 20% of the patients with ACS reported a previous MI or PCI.

Coronary artery disease patients with previous MI were more frequent in the individual treatment groups than in the placebo group, representing a potential imbalance among groups ($P = 0.12$; Fisher's exact test). As for the other baseline demographics and clinical characteristics in both patient populations, no group-specific trend was noted.

Overall, there was a low incidence of CURE bleeding in both groups of patients (*Table 2*). In ACS patients, major life-threatening bleeding was seen in two patients in the placebo group (operative haemorrhage and retroperitoneal haematoma), but in none of the patients given E5555. No patient experienced major non-life-threatening bleeding. Minor bleeding was seen only in one patient in the 200 mg E5555 group (vessel puncture site haematoma).

In CAD patients, only two patients, one in the 100 mg E5555 group and the other in the 200 mg E5555 group, experienced CURE major and minor bleeds, respectively. The specific proportions of patients with such an event were as follows: placebo: 0.0% (0/66 patient); 50 mg: 0.0% (0/63); 100 mg: 1.5% (1/66); and 200 mg: 1.5% (1/68). No significant trend was observed in dose–response profiles in this category of bleeding ($P = 0.38$; Cochran–Armitage test).

There were 45 and 22 patients who experienced any TIMI bleeding (i.e. in any category including TIMI minimal bleeding) in the ACS and CAD studies, respectively (*Table 3*). TIMI bleeds due to invasive procedures were 26 of 45 in ACS and 0 of 22 in CAD patients, thus occurring in early phase (most of the events occurred within 15 days). There was a numerical increase in 'any' TIMI bleeding with the E5555 200 mg dose (ACS: 16.4% placebo vs. 23.0% E5555, $P = 0.398$; CAD: 4.5% placebo vs. 13.2% E5555, $P = 0.081$) as shown in *Figure 3*.

In ACS patients, the incidence rates of TIMI bleeding were as follows: placebo: 10 of 61 patients (16.4%); 50 mg: 8 of 54 (14.8%); 100 mg: 13 of 65 (20.0%); 200 mg: 14 of 61 (23.0%); the 100 and 200 mg groups showed a slightly higher rate than the placebo group. Although there was a numerical increase in bleeding in patients treated with higher doses of E5555, no significant dose-dependent difference was observed for the incidence of TIMI bleeding ($P = 0.27$; Cochran–Armitage test) and no significant difference was observed between placebo and all active combined groups ($P = 0.61$).

In all groups, no patient experienced TIMI major bleeding. TIMI minor bleeding was seen in 2 of 65 (3.1%) in the 100 mg E5555

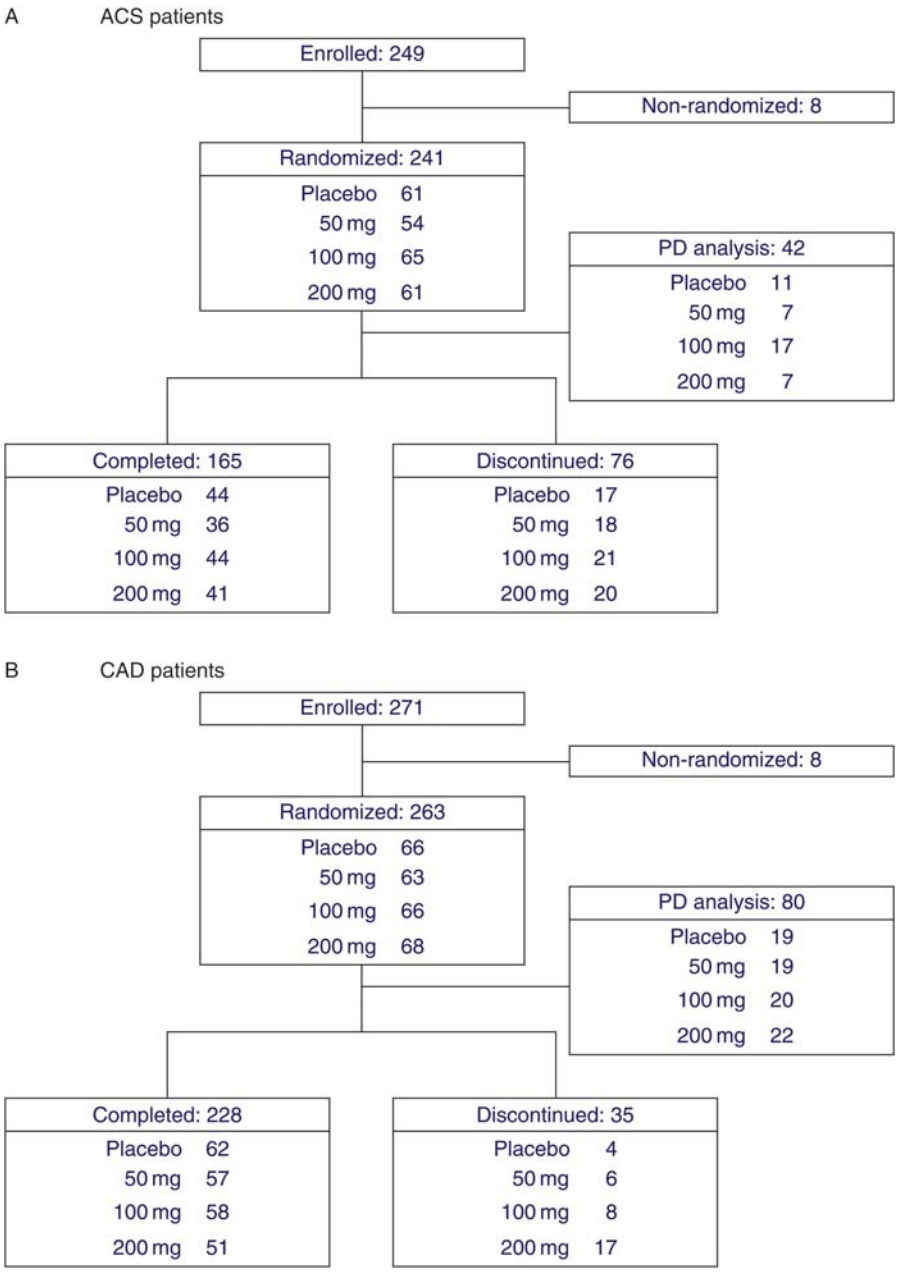

Figure 2 Study protocol. We have conducted two Phase II studies for acute coronary syndrome and high-risk coronary artery disease separately. Study protocol for acute coronary syndrome is shown in (*A*), whereas protocol for high-risk coronary artery disease is shown in (*B*). Study protocols for the two studies are similar, but differ in baseline use of antiplatelets and exposure period of the study drug. Details are described in the Methods section.

group (vessel puncture site haematoma and haematuria) and 1 of 61 (1.6%) in the 200 mg E5555 group (vessel puncture site haematoma); no patients in the placebo group or the 50 mg E5555 group experienced such an event.

In CAD patients, the rates of TIMI bleeding were as follows: placebo: 3 of 66 (4.5%); 50 mg: 5 of 63 (7.9%); 100 mg: 5 of 66 (7.6%); and 200 mg: 9 of 68 (13.2%). A trend was observed in dose–response profiles ($P = 0.086$; Cochran–Armitage test). Each E5555 group showed a higher incidence than the placebo group. However, no significant difference was observed between placebo and all active combined groups ($P = 0.22$).

In all groups, no patient experienced TIMI-defined major bleeding. The occurrence of TIMI minor bleeding was limited to one patient (gastrointestinal haemorrhage) in the 100 mg group.

The proportions of patients with CEC-adjudicated MACEs during the treatment period in ACS and CAD studies are shown (*Figure 4*). In ACS patients, the occurrence of MACEs was 4 of 61 (6.6%), 4 of 54 (7.4%), 3 of 65 (4.6%), and 2 of 61 (3.3%) in

Table 1 Baseline demographic and clinical characteristic

Treatment group	Patients with ACS					Patients with CAD				
	Placebo(n = 61)	E5555				Placebo (n = 66)	E5555			
		All (n = 180)	50 mg (n = 54)	100 mg (n = 65)	200 mg (n = 61)		All (n = 197)	50 mg (n = 63)	100 mg (n = 66)	200 mg (n = 68)
Age (mean ± SD)	64.5 ± 9.8	65.2 ± 8.5	65.4 ± 8.0	66.3 ± 8.5	63.8 ± 8.9	65.4 ± 7.2	66.9 ± 7.2	66.8 ± 7.5	66.7 ± 7.4	67.1 ± 6.8
Male, *n* (%)	50 (82.0)	144 (80.0)	47 (87.0)	52 (80.0)	45 (73.8)	55 (83.3)	175 (88.8)	58 (92.1)	59 (89.4)	58 (85.3)
Weight, kg (mean ± SD)	66.0 ± 12.2	64.2 ± 12.3	63.5 ± 9.4	63.8 ± 12.5	65.3 ± 14.4	66.3 ± 11.8	66.4 ± 9.8	65.6 ± 9.2	66.4 ± 10.4	67.2 ± 9.6
Previous ACS, *n* (%)	9 (14.8)	38 (21.1)	14 (25.9)	13 (20.0)	11 (18.0)	41 (62.1)	147 (74.6)	48 (76.2)	52 (78.8)	47 (69.1)
Previous PCI, *n* (%)	7 (11.5)	38 (21.1)	13 (24.1)	13 (20.0)	12 (19.7)	55 (84.6)	165 (83.8)	52 (82.5)	53 (80.3)	60 (88.2)
Previous CABG, *n* (%)	2 (3.3)	4 (2.2)	3 (5.6)	0 (0.0)	1 (1.6)	11 (16.7)	37 (18.8)	13 (20.6)	15 (22.7)	9 (13.2)
TIA/stroke, *n* (%)	4 (6.6)	16 (8.9)	7 (13.0)	4 (6.2)	5 (8.2)	13 (19.7)	29 (14.7)	9 (14.3)	11 (16.7)	9 (13.2)
Diabetes, *n* (%)	17 (27.9)	65 (36.1)	22 (40.7)	21 (32.3)	22 (36.1)	63 (95.5)	186 (94.4)	60 (95.2)	62 (93.9)	64 (94.1)
Hypertension, *n* (%)	45 (73.8)	141 (78.3)	42 (77.8)	53 (81.5)	46 (75.4)	53 (80.3)	162 (82.2)	50 (79.4)	52 (78.8)	60 (88.2)
Dyslipidaemia, *n* (%)	42 (68.9)	142 (78.9)	39 (72.2)	52 (80.0)	51 (83.6)	60 (90.9)	177 (89.8)	56 (88.9)	60 (90.9)	61 (89.7)
Aspirin, *n* (%)	61 (100)	175 (97.2)	52 (96.3)	63 (96.9)	60 (98.4)	66 (100)	197 (100)	63 (100)	66 (100)	68 (100)
Thienopyridine, *n* (%)	58 (95.1)	167 (92.8)	51 (94.4)	62 (95.4)	54 (88.5)	26 (39.4)	83 (42.1)	21 (33.3)	30 (45.5)	32 (47.1)
PCI in study period	55 (90.2)	155 (86.1)	46 (85.2)	54 (83.1)	55 (90.2)	—	—	—	—	—

ACS, acute coronary syndrome; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; TIA, transient ischaemic attack.

Table 2 CURE bleeding

Treatment group	Patients with ACS					Patients with CAD				
	Placebo ($n = 61$)	E5555				Placebo ($n = 66$)	E5555			
		All ($n = 180$)	50 mg ($n = 54$)	100 mg ($n = 65$)	200 mg ($n = 61$)		Al ($n = 197$)	50 mg ($n = 63$)	100 mg ($n = 66$)	200 mg ($n = 68$)
Any CURE bleeding	2 (3.3)	1 (0.6) ($P = 0.125$)*	0 (0.0) ($P = 0.221$)	0 (0.0) ($P = 0.151$)	1 (1.6) ($P = 0.681$)	0 (0.0)	2 (1.0) ($P = 0.476$)	0 (0.0) (—)	1 (1.5) ($P = 0.528$)	1 (1.5) ($P = 0.514$)
Major bleeding	2 (3.3)	0 (0.0) ($P = 0.021$)	0 (0.0) ($P = 0.221$)	0 (0.0) ($P = 0.151$)	0 (0.0) ($P = 0.209$)	0 (0.0)	1 (0.5) ($P = 0.728$)	0 (0.0) (—)	1 (1.5) ($P = 0.528$)	0 (0.0) (—)
Life-threatening	2 (3.3)	0 (0.0) ($P = 0.021$)	0 (0.0) ($P = 0.221$)	0 (0.0) ($P = 0.151$)	0 (0.0) ($P = 0.209$)	0 (0.0)	1 (0.5) ($P = 0.728$)	0 (0.0) (—)	1 (1.5) ($P = 0.528$)	0 (0.0) (—)
Non-life-threatening	0 (0.0)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)
Minor bleeding	0 (0.0)	1 (0.6) ($P = 0.727$)	0 (0.0) (—)	0 (0.0) (—)	1 (1.6) ($P = 0.528$)	0 (0.0)	1 (0.5) ($P = 0.728$)	0 (0.0) (—)	0 (0.0) (—)	1 (1.5) ($P = 0.514$)
Cochran–Armitage test**	$P = 0.702$					$P = 0.379$				

*P-values vs. placebo group.
**P-values show the dose relationship of any CURE bleeding.

Table 3 TIMI bleeding

Treatment group	Patients with ACS					Patients with CAD				
	Placebo ($n = 61$)	E5555				Placebo ($n = 66$)	E5555			
		All ($n = 180$)	50 mg ($n = 54$)	100 mg ($n = 65$)	200 mg ($n = 61$)		All ($n = 197$)	50 mg ($n = 63$)	100 mg ($n = 66$)	200 mg ($n = 68$)
Any TIMI bleeding	10 (16.4)	35 (19.4) ($P = 0.609$)*	8 (14.8) ($P = 0.850$)	13 (20.0) ($P = 0.692$)	14 (23.0) ($P = 0.398$)	3 (4.5)	19 (9.6) ($P = 0.219$)	5 (7.9) ($P = 0.530$)	5 (7.6) ($P = 0.531$)	9 (13.2) ($P = 0.081$)
Major bleeding	0 (0.0)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)	0 (0.0) (—)
Minor bleeding	0 (0.0)	3 (1.7) ($P = 0.380$)	0 (0.0) (—)	2 (3.1) ($P = 0.222$)	1 (1.6) ($P = 0.528$)	0 (0.0)	1 (0.5) ($P = 0.728$)	0 (0.0) (—)	1 (1.5) ($P = 0.528$)	0 (0.0) (—)
Minimal bleeding	10 (16.4)	32 (17.8) ($P = 0.824$)	8 (14.8) ($P = 0.850$)	11 (16.9) ($P = 0.998$)	13 (21.3) ($P = 0.526$)	3 (4.5)	18 (9.1) ($P = 0.244$)	5 (7.9) ($P = 0.530$)	4 (6.1) ($P = 0.791$)	9 (13.2) ($P = 0.081$)
With medical attention	4 (6.6)	6 (3.3) ($P = 0.303$)	1 (1.9) ($P = 0.252$)	1 (1.5) ($P = 0.173$)	4 (6.6) ($P = 1.000$)	1 (1.5)	2 (1.0) ($P = 0.832$)	1 (1.6) ($P = 1.000$)	0 (0.0) ($P = 0.528$)	1 (1.5) ($P = 1.000$)
Without medical attention	6 (9.8)	26 (14.4) ($P = 0.371$)	7 (13.0) ($P = 0.671$)	10 (15.4) ($P = 0.514$)	9 (14.8) ($P = 0.531$)	2 (3.0)	16 (8.1) ($P = 0.171$)	4 (6.3) ($P = 0.530$)	4 (6.1) ($P = 0.531$)	8 (11.8) ($P = 0.056$)
Cochran–Armitage test**	$P = 0.266$					$P = 0.086$				

Medical attention: any bleeding that requires medical treatment, surgical treatment, or laboratory evaluation and does not meet criteria for major or minor bleeding.
*P-values vs. placebo group.
**P-values show the dose relationship of any TIMI bleeding.

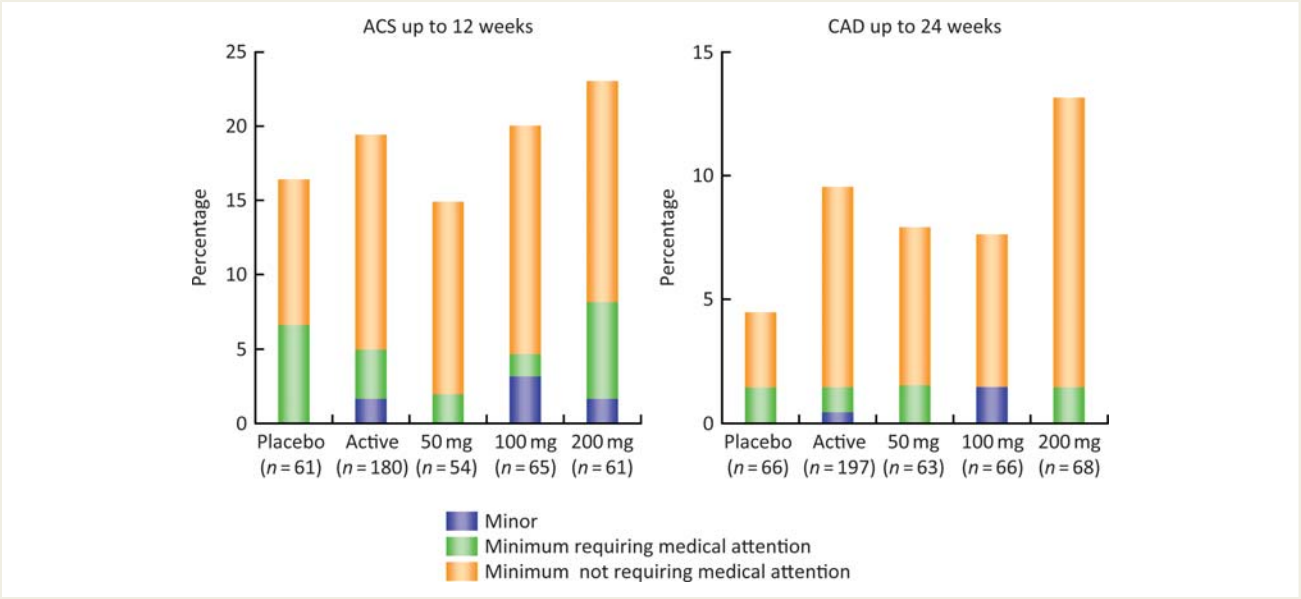

Figure 3 CEC-adjudicated TIMI bleeding by category. CEC-adjudicated TIMI bleeding in placebo, E5555 pooled, and each dose tested of E5555, as shown. Any TIMI bleeding, including minimal bleeding not requiring medical attention, occurred more in patients treated by E5555. However, TIMI bleeding requiring medical attention or more severe bleeding did not increase even when patients were treated by E5555. Details are described in the Results section.

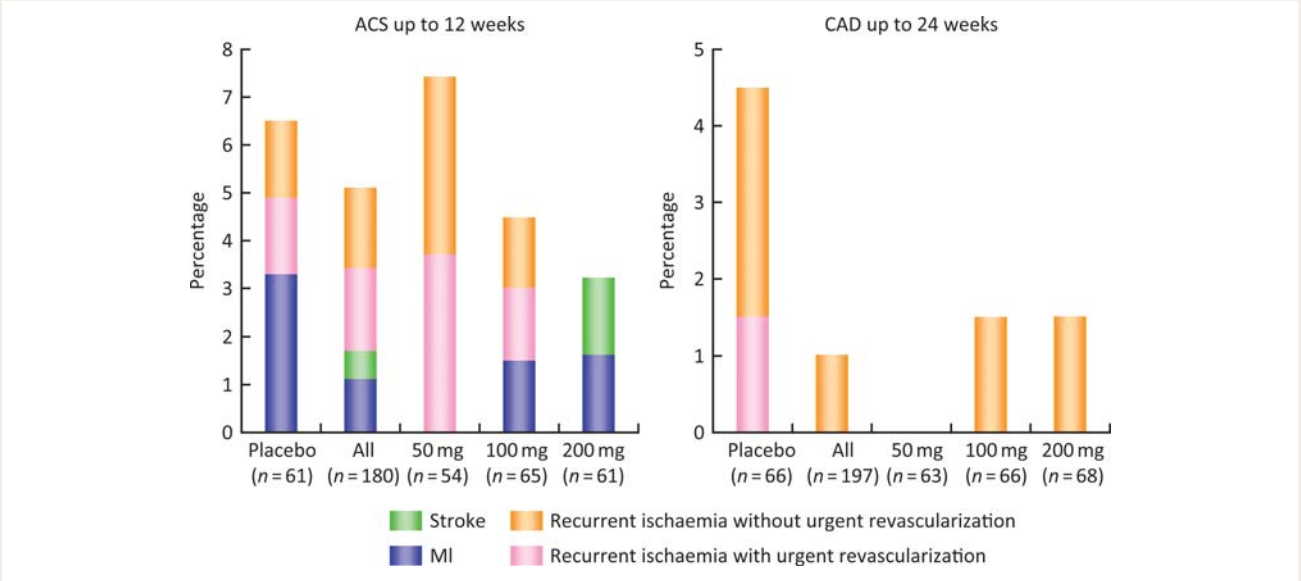

Figure 4 CEC-adjudicated MACEs by category. CEC-adjudicated each category of major cardiovascular adverse events in placebo, E5555 pooled, and each dose tested, as shown. Details are described in the Results section.

the placebo, 50, 100, and 200 mg E5555 groups, respectively. The percentage occurrence of MACEs in the active combined group and placebo group (5.0 vs. 6.6%, respectively; relative risk 0.76; 95% CI 0.257–2.606) was not significantly different ($P = 0.73$). The most frequent MACE was recurrent ischaemia, and no cardiovascular death occurred in any group. The second most frequent MACE was MI, with occurrence of 2 of 61 (3.3%), 0 of 54 (0.0%), 1 of 65 (1.5%), and 1 of 61 (1.6%) in the placebo, 50, 100, and 200 mg E5555 groups, respectively. Ischaemic stroke induced by embolism was seen only in the 200 mg E5555 group in 1 of 61 patients (1.6%).

In CAD patients, the occurrence of MACEs was 3 of 66 (4.5%), 0 of 63 (0.0%), 1 of 66 (1.5%), and 1 of 68 (1.5%) in the placebo, 50, 100, and 200 mg E5555 groups, respectively. The rate of

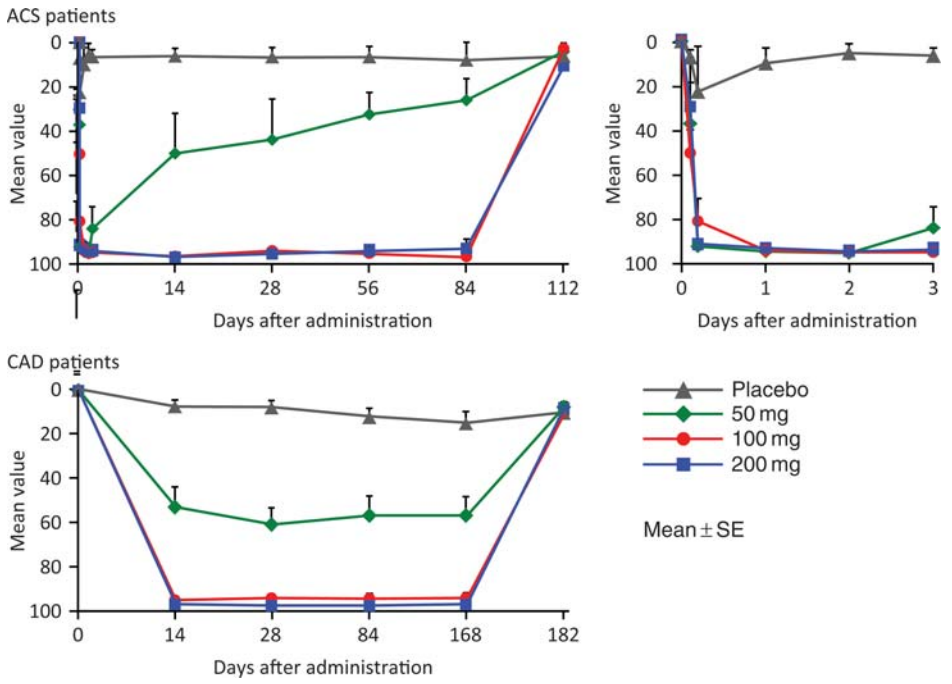

Figure 5 Inhibition of platelet aggregation induced thrombin receptor-activating peptide (TRAP) at a final concentration of 15 μM is shown in both acute coronary syndrome and coronary artery disease patients. In the upper right panel, detailed changes in the inhibition of TRAP-induced platelet aggregation within 3 days after drug administration are shown. Each point represents mean ± SD of sample tested. Details are described in the Results section.

MACEs was numerically lower in the active combined group than in the placebo group (1.0 vs. 4.5%, respectively; relative risk 0.22; 95% CI 0.041–1.109), although this was not statistically significant ($P = 0.066$). All MACEs were recurrent ischaemia events and no cardiovascular death, MI, or stroke occurred in any group.

The PAI induced by 15 μM TRAP is shown in *Figure 5*. In both studies, the mean PAI in patients at trough treated with 100 or 200 mg E5555 was over 90%, and the mean PAI at trough in patients treated with 50 mg was 20–50% in ACS patients and 50–60% in CAD patients. In ACS patients treated with E5555, the mean PAI reached >80% at 3–6 h after administration of the 400 mg E5555 loading dose. In all of the E5555 treatment groups, the mean PAI markedly decreased after the completion of study drug administration, and the effect on PAI completely disappeared at 2 weeks after the completion of study drug.

The incidences of adverse events, serious adverse events, and higher frequency adverse events are shown in *Tables 4* and *5*. Overall, there were significantly more treatment-related adverse events in patients treated with E5555 than in patients receiving placebo (44.4 vs. 27.9%, respectively, in ACS patients, $P = 0.024$; 32.0 vs. 13.6%, respectively, in CAD patients, $P = 0.003$).

In both studies, the most common adverse event related to study drug was abnormal liver function. For individual hepatic function parameters (ALT and AST), the proportion of patients with an abnormally high level was calculated for all groups using the FDA guideline 'Guidance for Industry, Drug-induced Liver Injury: Premarketing Clinical Evaluation' (*Figure 6*).

In ACS patients, the proportions of patients with the ALT level >3×ULN were 0.0% (0/60), 0.0% (0/54), 6.2% (4/65), and 14.8% (9/61) in the placebo, 50, 100 and 200 mg E5555 groups, respectively (in the 200 mg E5555 group, $P = 0.001$). In the placebo and 50 mg E5555 groups, no patient met this criterion. There was a significant increase for the rate of ALT elevation in the active combined group vs. the placebo group (7.2 vs. 0.0%, respectively; $P = 0.034$). The proportions of patients with the AST level >3× ULN were 1.7% (1/60), 0.0% (0/54), 1.5% (1/65), and 9.8% (6/61) in the placebo, 50, 100, and 200 mg E5555 groups, respectively (in the 200 mg E5555 group, relative risk 5.902; 95% CI 0.938–75.547, $P = 0.062$). In the 50 mg E5555 group, no patient met this criterion. The rate of AST elevation in the active combined group vs. the placebo group was 3.9 vs. 1.7%, respectively; relative risk 2.333; 95% CI 0.380–29.538, $P = 0.447$.

In CAD patients, the proportions of patients with an ALT level >3× ULN were 0.0% (0/66), 1.6% (1/63), 1.5% (1/66), and 11.8% (8/68) in the placebo, 50, 100, and 200 mg E5555 groups, respectively (in the 200 mg E5555 group, $P = 0.004$). In the placebo group, no patient met this criterion. There was a non-significant increase for the rate of ALT elevation in the active combined group vs. the placebo group (5.1 vs. 0.0%, respectively; $P = 0.061$). The proportions of patients with an AST level >3× ULN were 0.0% (0/66), 1.6% (1/63), 0.0% (0/66), and 8.8% (6/68) in the placebo, 50, 100, and 200 mg E5555 groups, respectively (in the 200 mg E5555 group, $P = 0.012$). In the 100 mg and placebo groups, no patient met this criterion. The rate of AST elevation in the active

Table 4 Incidence of overall adverse events

Treatment group	Patients with ACS					Patients with CAD				
	Placebo ($n = 61$)	E5555				Placebo ($n = 66$)	E5555			
		All ($n = 180$)	50 mg ($n = 54$)	100 mg ($n = 65$)	200 mg ($n = 61$)		All ($n = 197$)	50 mg ($n = 63$)	100 mg ($n = 66$)	200 mg ($n = 68$)
Adverse events (AE)	53 (86.9)	168 (93.3) ($P = 0.175$)	48 (88.9) ($P = 0.782$)	60 (92.3) ($P = 0.386$)	60 (98.4) ($P = 0.032$)	48 (72.7)	147 (74.6) ($P = 0.748$)	44 (69.8) ($P = 0.845$)	48 (72.7) ($P = 1.000$)	55 (80.9) ($P = 0.308$)
Treatment- related AE	17 (27.9)	80 (44.4) ($P = 0.024$)	17 (31.5) ($P = 0.687$)	34 (52.3) ($P = 0.006$)	29 (47.5) ($P = 0.039$)	9 (13.6)	63 (32.0) ($P = 0.003$)	17 (27.0) ($P = 0.078$)	14 (21.2) ($P = 0.358$)	32 (47.1) ($P < 0.001$)
Serious AE	9 (14.8)	26 (14.4) ($P = 1.000$)	9 (16.7) ($P = 0.802$)	8 (12.3) ($P = 0.796$)	9 (14.8) ($P = 1.000$)	7 (10.6)	18 (9.1) ($P = 0.808$)	4 (6.3) ($P = 0.531$)	6 (9.1) ($P = 1.000$)	8 (11.8) ($P = 1.000$)
Treatment-related serious AE	3 (4.9)	9 (5.0) ($P = 1.000$)	1 (1.9) ($P = 0.621$)	3 (4.6) ($P = 1.000$)	5 (8.2) ($P = 0.717$)	1 (1.5)	5 (2.5) ($P = 1.000$)	1 (1.6) ($P = 1.000$)	1 (1.5) ($P = 1.000$)	3 (4.4) ($P = 0.619$)

Data are expressed as number of patients (% total). *P*-values vs. placebo group.

Table 5 Incidence of frequent adverse events

Treatment group	Patients with ACS					Patients with CAD				
	Placebo ($n = 61$)	E5555				Placebo ($n = 66$)	E5555			
		All ($n = 180$)	50 mg ($n = 54$)	100 mg ($n = 65$)	200 mg ($n = 61$)		All ($n = 197$)	50 mg ($n = 63$)	100 mg ($n = 66$)	200 mg ($n = 68$)
Nasopharyngitis	10 (16.4)	17 (9.4) ($P = 0.159$)	8 (14.8) ($P = 1.000$)	4 (6.2) ($P = 0.090$)	5 (8.2) ($P = 0.269$)	14 (21.2)	53 (26.9) ($P = 0.416$)	16 (25.4) ($P = 0.677$)	19 (28.8) ($P = 0.421$)	18 (26.5) ($P = 0.545$)
Hepatic function disorder	7 (11.5)	42 (23.3) ($P = 0.064$)	5 (9.3) ($P = 0.767$)	19 (29.2) ($P = 0.015$)	18 (29.5) ($P = 0.023$)	1 (1.5)	20 (10.2) ($P = 0.032$)	2 (3.2) ($P = 0.613$)	5 (7.6) ($P = 0.207$)	13 (19.1) ($P = 0.001$)
Insomnia	10 (16.4)	17 (9.4) ($P = 0.159$)	4 (7.4) ($P = 0.164$)	5 (7.7) ($P = 0.171$)	8 (13.1) ($P = 0.799$)	3 (4.5)	1 (0.5) ($P = 0.049$)	0 (0.0) ($P = 0.244$)	1 (1.5) ($P = 0.619$)	0 (0.0) ($P = 0.116$)
Upper respiratory tract inflammation	1 (1.6)	1 (0.6) ($P = 0.442$)	0 (0.0) ($P = 1.000$)	0 (0.0) ($P = 0.484$)	1 (1.6) ($P = 1.000$)	3 (4.5)	12 (6.1) ($P = 0.767$)	3 (4.8) ($P = 1.000$)	7 (10.6) ($P = 0.324$)	2 (2.9) ($P = 0.678$)
Headache	5 (8.2)	21 (11.7) ($P = 0.633$)	6 (11.1) ($P = 0.753$)	5 (7.7) ($P = 1.000$)	10 (16.4) ($P = 0.269$)	3 (4.5)	4 (2.0) ($P = 0.372$)	2 (3.2) ($P = 1.000$)	0 (0.0) ($P = 0.244$)	2 (2.9) ($P = 0.678$)
Constipation	9 (14.8)	27 (15.0) ($P = 1.000$)	4 (7.4) ($P = 0.250$)	11 (16.9) ($P = 0.810$)	12 (19.7) ($P = 0.632$)	1 (1.5)	4 (2.0) ($P = 1.000$)	1 (1.6) ($P = 1.000$)	I (1.5) ($P = 1.000$)	2 (2.9) ($P = 1.000$)
Back pain	10 (16.4)	24 (13.3) ($P = 0.530$)	7 (13.0) ($P = 0.793$)	7 (10.8) ($P = 0.437$)	10 (16.4) ($P = 1.000$)	4 (6.1)	6 (3.0) ($P = 0.275$)	4 (6.3) ($P = 1.000$)	2 (3.0) ($P = 0.680$)	0 (0.0) ($P = 0.056$)
Chest discomfort	11 (18.0)	27 (15.0) ($P = 0.549$)	10 (18.5) ($P = 1.000$)	6 (9.2) ($P = 0.194$)	11 (18.0) ($P = 1.000$)	1 (1.5)	3 (1.5) ($P = 1.000$)	2 (3.2) ($P = 0.613$)	1 (1.5) ($P = 1.000$)	0 (0.0) ($P = 0.492$)
Chest pain	8 (13.1)	19 (10.6) ($P = 0.639$)	3 (5.6) ($P = 0.213$)	9 (13.8) ($P = 1.000$)	7 (11.5) ($P = 1.000$)	2 (3.0)	3 (1.5) ($P = 0.601$)	1 (1.6) ($P = 1.000$)	1 (1.5) ($P = 1.000$)	1 (1.5) ($P = 0.616$)
Pyrexia	3 (4.9)	23 (12.8) ($P = 0.098$)	5 (9.3) ($P = 0.471$)	10 (15.4) ($P = 0.077$)	8 (13.1) ($P = 0.204$)	1 (1.5)	2 (1.0) ($P = 1.000$)	0 (0.0) ($P = 1.000$)	0 (0.0) ($P = 1.000$)	2 (2.9) ($P = 1.000$)

Data are expressed as number of patients (% total). *P*-values vs. placebo group.

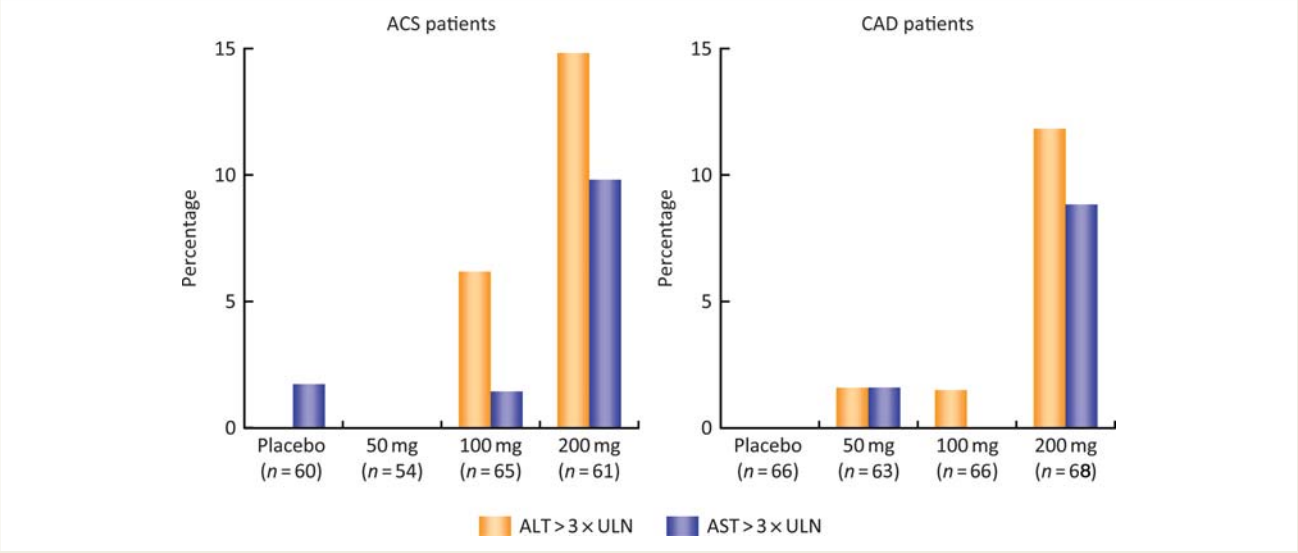

Figure 6 Proportions of patients with high level of hepatic function parameter. Proportions of patients who experienced hepatic dysfunction defined by alanine aminotransferase and aspartate aminotransferase three times more than the upper limit of normal are shown. Details are described in the Results section.

combined group vs. the placebo group was 3.6 vs. 0.0%, respectively; $P = 0.133$.

In detail, 6 of 7 in placebo, 5 of 5 at 50 mg, 19 of 19 at 100 mg, and 16 of 18 cases of hepatic disorder at 200 mg occurred in ACS patients treated either by ticlopidine or clopidogrel. On the other hand, 0 of 1 in placebo, 0 of 2 at 50 mg, 0 of 5 at 100 mg, and 4 of 13 cases at 200 mg occurred in CAD patients who were also treated by thienopyridine.

There was no patient who experienced elevation of AST or ALT $>3\times$ ULN with elevation of total bilirubin $>2\times$ ULN.

There were also dose-related effects on QTcF noted with E5555. In ACS patients at Week 12 LOCF, the changes in QTcF from baseline were -8.3 ± 3.6, -5.1 ± 3.4, -6.3 ± 3.1, and -0.9 ± 4.3 ms, in the placebo, 50, 100, and 200 mg E5555 groups, respectively (i.e. the QTcF shortened from baseline to 12 weeks in placebo in these ACS patients, but shortened less in the treated groups). There was a trend towards a difference between the placebo and 200 mg E5555 groups ($P = 0.066$), and there was a trend towards statistically significant dose-dependent QTcF prolongation ($P = 0.074$; Jonckheere–Terpstra's test). In CAD patients at Week 24 LOCF, the changes from baseline in QTcF were 1.4 ± 2.4, 4.1 ± 2.9, 6.1 ± 2.2, and 6.3 ± 2.9 ms in the placebo, 50, 100, and 200 mg E5555 groups, respectively. There were significant prolongations in the 100 mg ($P = 0.015$) and 200 mg ($P = 0.037$) groups in comparison with the placebo group. There was a statistically significant dose-dependent QTcF prolongation ($P = 0.026$; Jonckheere–Terpstra's test).

Discussion

In these two multicentre, randomized, double-blind, placebo-controlled Phase II studies of Japanese patients, the orally active PAR-1 antagonist E5555 showed a numerical increase in 'nuisance' bleeding events dose-dependently, but did not increase clinically significant bleeding events in ACS or CAD. There was a higher incidence of any bleeding events in ACS patients when compared with CAD, which might be due to more invasive procedures or higher use of thienopyridines, but might partly be due to the loading dose of E5555 used in the ACS trial. Clinically significant bleeding events were relatively uncommon in patients receiving concomitant aspirin and thienopyridine, as was the case in the majority of the patients in both studies.

The combined E5555 treatment group in the ACS patients, and all individual E5555 treatment groups in the CAD patients, showed a numerically lower incidence of MACEs than their respective placebo groups. These data highlight the potential of E5555 to reduce ischaemic events in ACS and CAD patients. However, the incidence of MACEs in all groups was low and the study was not powered to demonstrate significant effects of E5555 vs. placebo on MACEs. Therefore, additional, adequately powered studies are required to evaluate the effects of E5555 on MACEs more fully. Of note, no CV deaths and a low incidence of MACEs were observed in both studies, and it is generally known that CV death and ischaemic event rates would be low in Japan.[3,24]

In patients with ACS, the E5555 loading dose (400 mg) rapidly inhibited platelet aggregation, and this effect was maintained with 100 and 200 mg daily up to Week 12. E5555 showed >90% inhibition of the platelet thrombin receptor at 100 and 200 mg. Moreover, the effect of PAI disappeared after the completion or discontinuation of E5555 administration. This suggests that the PAI effect of E5555 may be reversible and related to its plasma concentration. E5555 inhibited platelet aggregation for up to 24 weeks in patients with CAD at all doses tested. These data demonstrate a significant pharmacodynamic effect of E5555 associated with inhibition of platelet aggregation, and this effect was both rapid in onset and sustained.

E5555 was more likely to cause enzyme elevations in liver function tests at a dose of 200 mg than at lower doses. In CAD patients especially, the discontinuation rate in the 200 mg E5555 group was higher than the placebo group (25.0 vs. 6.1%, respectively), and this was due to the occurrence of liver dysfunction. The incidence of liver function test abnormalities in ACS patients was much higher than in CAD patients. Most ACS patients were treated by thienopyridines. Some ACS patients who experienced liver function abnormalities may be due to the treatment by thienopyridine because the incidence of liver function abnormalities in the placebo group was much higher than in the placebo group in CAD patients. Regardless, our results demonstrating dose-dependent increases in hepatic dysfunction with the use of E5555 raise caution of hepatic disorders induced by E5555. Since the number of patients exposed to E5555 in the studies described in this manuscript is small, any future studies would need to consider Hy's law and the potential for serious hepatic toxicity.[25] There was also a statistically significant QT prolongation in the groups receiving the two higher E5555 doses compared with the placebo group. The degree of QT prolongation and liver dysfunction with the two higher doses of E5555 should be carefully considered in any future studies.

Despite rigorous treatment with currently available antiplatelet agents, patients with ACS and CAD continue to experience major atherothrombotic events, indicating that there is still a need for new antiplatelet therapies. The current standard of care for ACS patients is dual antiplatelet therapy with aspirin and clopidogrel, although studies show that newer $P2Y_{12}$ inhibitors, including prasugrel and ticagrelor, are more effective than clopidogrel.[26,27] Limitations of clopidogrel include relatively slow onset of effect, low PAI in many patients, inter-individual variability in response, and irreversible $P2Y_{12}$ binding that prevents rapid offset of effect. Novel antiplatelet agents targeting the thrombin-specific pathway, such as the PAR-1 antagonist E5555, have great potential to fulfil some of the current limitations of dual antiplatelet therapy by providing rapid onset of action, enhanced inhibition of platelet aggregation, as well as targeting an additional platelet activation pathway. This may allow the therapy to be added on to existing treatments to further minimize the risk of atherothrombotic events without significantly increasing bleeding rates. Recently, dabigatran, an oral direct thrombin inhibitor, showed a lower rate of stroke compared with warfarin in atrial fibrillation patients.[28]

Effective antiplatelet treatment is a balance between atherothrombotic risk and bleeding risk. It has been clearly demonstrated that greater platelet inhibition may produce better clinical outcome by reducing ischaemic events, but at the risk of higher bleeding when targeting $P2Y_{12}$.[26,29] It is well known that patients deficient in $P2Y_{12}$ have a bleeding disorder,[30] whereas no PAR-1 deficient patients were reported as having a bleeding disorder. Our study showed that there was an apparent dose–response in bleeding and adverse events, including elevation of enzyme levels in liver function tests and QTcF, with E5555 compared with placebo. Platelet inhibition measured by the TRAP method was about 50% with 50 mg E5555, whereas it was >90% with 100 and 200 mg E5555.

These studies were conducted as the first investigation and exploratory pilot studies with Japanese patients. Therefore, these studies were limited in their ability to detect any statistical differences in safety and efficacy between the placebo and E5555 groups or combined active group. For that reason, no adjustments were made for multiple comparisons.

Conclusion

In conclusion, E5555 added to standard antiplatelet therapy may have potential for reducing MACEs in patients with ACS or high-risk CAD, without increasing the incidence of clinically significant bleeding. The issues of QT prolongation and liver dysfunction with E5555 would need to be considered in any potential future studies. Nevertheless, antagonism of the PAR-1 receptor appears to be an attractive pathway for the treatment of atherothrombosis.

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Appendix 1

Classification of bleeding severity (CURE and TIMI criteria)

Severity	CURE criteria		TIMI criteria
	Type	Description	
Major	Life-threatening	Fatal Symptomatic intracranial haemorrhage Causes haemoglobin drop >5 g/dL Necessitates transfusion of ≥4 U of packed red blood cell for blood transfusion (PRBC) Causes hypotension requiring administration of an inotropic agent or a surgical intervention	(1) If it is intracranial, or (2) clinically significant overt signs of haemorrhage associated with a drop in haemoglobin of >5 g/dL (or, when haemoglobin is not available, an absolute drop in haematocrit of >15%) (3) If CABG related: fatal bleeding or perioperative intracranial bleeding or reoperation following closure of the sternotomy incision for the purpose of controlling bleeding or transfusion of >5 U of whole blood or PRBC within a 48 h period (cell saver transfusion will not be counted in calculations of blood products) or chest tube output >2 L within a 24 h period
	Non-life-threatening	Necessitates transfusion of 2–3 U of PRBC Intraocular haemorrhage Causes other significant disability	
Minor		Other haemorrhages that lead to the interruption of the study drug	Any clinically overt sign of haemorrhage (including imaging) that is associated with a fall in haemoglobin of 3 to ≤5 g/dL (or, when haemoglobin is not available, a fall in haematocrit of 9 to ≤15%)
Minimal		None	Any clinically overt sign of haemorrhage (including imaging) that is associated with a fall in haemoglobin <3 g/dL (or, when haemoglobin is not available, a fall in haematocrit of <9%)

Appendix 2

J-LANCELOT Investigators

ACS study

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CAD study

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