

Safety and Tolerability, Pharmacokinetics, and Pharmacodynamics of ACT017, an Antiplatelet GPVI (Glycoprotein VI) Fab

First-in-Human Healthy Volunteer Trial

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Objective—ACT017 is a novel, first in class, therapeutic antibody to platelet GPVI (glycoprotein VI) with potent and selective antiplatelet effects. This first-in-human, randomized, placebo-controlled phase 1 study was conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of ACT017 in healthy subjects.

Approach and Results—Six cohorts of 8 healthy male and female subjects each received ascending single doses of ACT017 (n=6) or placebo (n=2) as a 6-hour intravenous infusion, with ¼ of the total dose administered within 15 minutes and the rest of the dose (¾ of the total dose) administered within 5 hours and 45 minutes. The 6 investigated doses ranged from 62.5 to 2000 mg. All doses of ACT017 were well tolerated, and no serious adverse events occurred during the study. None of the subjects reported an infusion site reaction. Template bleeding time was not affected in a clinically significant manner by any of the ACT017 doses. Plasma concentrations, determined by liquid chromatography-tandem mass spectrometry, increased linearly with the dose received as were the established pharmacokinetics values. There was no change in the platelet count, platelet GPVI expression assessed by flow cytometry, or plasma levels of soluble GPVI assessed by ELISA. In contrast, administration of ACT017 inhibited collagen-induced platelet aggregation measured by light transmission aggregometry on platelet-rich plasma, and the extent and duration of the effect were dose-dependent.

Conclusions—The novel antiplatelet agent ACT017 has consistent pharmacokinetic/pharmacodynamic properties and favorable safety and tolerability profiles warranting further clinical development.

Visual Overview—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2019;39:956-964. DOI: 10.1161/ATVBAHA.118.312314.)

Key Words: immunoglobulin Fab fragment ■ pharmacodynamics ■ pharmacokinetics ■ platelet aggregation inhibitors ■ platelet membrane glycoprotein ■ safety

Platelets are central to thrombus formation, the leading cause of global mortality estimated to account for 1 in 4 death worldwide in 2010.^{1,2} Thrombosis is associated with cardiovascular diseases (myocardial infarction, stroke, lower limb ischemia, venous thromboembolism) and with numerous pathologies, such as cancer (Trousseau syndrome) infections or inflammatory diseases. Antiplatelet drugs, such as aspirin, P2Y₁₂ antagonists, and GP (glycoprotein) IIb/IIIa inhibitors are of proven benefit in reducing the morbidity and mortality associated with arterial thrombosis.³ These agents are, therefore, the cornerstone of therapy for patients with acute coronary syndromes. However, these drugs all carry an inherent risk of bleeding that restricts their use in sensitive populations (eg, elderly) and when arterial thrombosis occurs in the cerebral territory.

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At present, the only acute treatment option available for ischemic stroke consists of revascularization with r-tPA (recombinant tissue-type plasminogen activator) and mechanical thrombectomy. Several large clinical trials have proven a strong clinical effect of complete recanalization achieved with mechanical thrombectomy combined with r-tPA in large vessel occlusion and severe ischemic stroke.⁴ However, significant limitations remain: (1) a substantial number of patients is not eligible for recanalization because of substantial extension or because of late presentation outside the accepted therapeutic window; (2) even complete recanalization does not systematically result in clinical improvement. The responsibility of platelets in the failure of thrombolysis/thrombectomy to restore

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Nonstandard Abbreviations and Acronyms

ADA	antidrug antibody
BT	bleeding time
GP	glycoprotein
r-tPA	recombinant tissue-type plasminogen activator

vascular patency is strongly suspected. However, and in contrast to what has been observed in interventional cardiology, blocking thromboxane A2 production by aspirin and even more blocking the integrin $\alpha\text{IIb}\beta\text{3}$ at the acute phase of ischemic stroke are associated with unacceptable hemorrhagic complications in humans and animals.⁵ There is, thus, a clear medical need for newer agents that can provide equivalent or even superior antithrombotic efficacy with an improved safety profile.

A still growing number of studies is highlighting potentially important differences between hemostasis and thrombosis, raising the prospect of developing new antiplatelet drugs that are not associated with bleeding.³ Platelet GPVI is a typical example of a platelet pathway that is dispensable for physiological hemostasis but critical for thrombus formation and growth.^{6,7} Patients with a GPVI deficiency present no or only a mild bleeding phenotype.^{8,9} In contrast, GPVI mediates platelet activation triggered by collagen and by fibrin including secretion, aggregation, and procoagulant activity.^{10,11} It is also involved in the fibrinogen-triggered outside-in signaling pathway.¹² As a consequence, GPVI is critical in the initiation and propagation phases of thrombus formation as evidenced in microfluidic assays *in vitro* and in different thrombosis models *in vivo*.¹³ The minor importance of GPVI for hemostasis can be explained by a greater role of other platelet agonists, such as von Willebrand factor and thrombin, that provide adapted compensatory responses. These observations, and the fact that GPVI is platelet-specific,¹⁴ suggest that pharmacological GPVI antagonists could have a specific and effective antithrombotic action combined with a minimal risk of bleeding.

Mouse monoclonal antibody 9O12 fragment (Fab 9O12) has proven to be specific for GPVI, to have a good affinity for GPVI, and to have a strong and reversible inhibitory efficacy without modifying GPVI expression *in vivo*.^{15–17} Recently, Fab9O12 has been successfully humanized. This new compound, ACT017, fully retains the specificity of the Fab 9O12 and its inhibitory properties.¹⁸

In nonclinical studies, ACT017 was well tolerated and safe when administered to nonhuman primates. The use of a 6-hour intravenous infusion of ACT017 at 8 mg/kg permitted a prolonged inhibition of platelet aggregation when compared with a 1-hour intravenous infusion at the same dose level, supporting the use of this administration mode in human. After completion of toxicology studies in cynomolgus monkeys showing no adverse effect at the highest dose of 80 mg/kg tested, a phase 1 study was conducted in healthy subjects. We report the safety, tolerability, pharmacokinetics, and pharmacodynamics of ACT017 in human healthy volunteers.

Materials and Methods

The data that support the findings of this study are available from the corresponding author and from Kristell Lebozec (kristell.lebozec@acticor-biotech.com) on reasonable request.

Study Objectives

The primary objectives for this first-in-human study were to evaluate the safety and tolerability of ACT017 administered as a 6-hour intravenous infusion, including assessment of bleeding time (BT). The secondary objectives were to assess ACT017 biological activity on collagen-induced platelet aggregation (pharmacodynamics) and to determine the plasma pharmacokinetics of ACT017.

The study was conducted by QPS Holdings LLC, the Netherlands, in compliance with the current revision of the Declaration of Helsinki, ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guideline for Good Clinical Practice, and current regulatory regulations (WMO [Medical Research Involving Human Subjects Act]). The clinical trial protocol, the subject information, and informed consent form were approved by the Independent Ethics Committee of the trial center. The competent authority of the Netherlands was notified, and a nonobjection statement was issued. All subjects provided written informed consent before any screening activity was conducted.

Study Design

This was a randomized, single ascending doses, placebo-controlled, double-blind phase 1 study. Because this was a first-in-human study in healthy subjects, in each treatment group, the first 2 subjects (1 on active and 1 on placebo) were dosed and monitored for safety and tolerability (including BT results) before the remaining subjects of the treatment group were dosed. Subsequently, the remaining 6 subjects were dosed no sooner than 48 hours after the first 2 subjects. Dose administration for subsequent treatment groups started no sooner than 7 days after the preceding dose was administered and proven to be well tolerated without any safety concerns.

Study Population

Eligible participants were healthy male and female volunteers (aged 18–65 years, inclusive) who had a body mass index between 18 and 30 kg/m² (inclusive) and who were in good physical and mental health as determined by medical history, physical examination, laboratory blood test, urine analysis, vital signs, and ECG examinations. Subjects with a history of hemorrhagic disease or venous or arterial thrombotic disease and subjects with a history or presence of any disorder with an increased risk of bleeding were excluded. Baseline laboratory test values were to be within reference ranges unless deemed not clinically significant by the investigator. Values for hemostasis and coagulation blood test, BT, and platelet aggregation had to be within normal limits. Exclusion criteria were: a creatinine clearance ≤ 60 mL/min (using the modification of diet in renal disease formula), history of alcohol or drug abuse, a positive test for hepatitis B, hepatitis C, HIV, or being a current smoker. Male and female subjects of childbearing potential had to use adequate contraception during and until 3 months after completion of the study. Subjects were screened within 4 weeks before dosing. Eligible subjects were admitted to the clinic on the day before administration of the study drug (day 1), for baseline assessments and to reconfirm eligibility. On day 1, all subjects were dosed as described below. Subjects were discharged from the clinic in the morning of day 3 after all scheduled assessments had been performed and if medically justified. A follow-up visit took place on day 7 \pm 2 days.

From 2 weeks or 5 half-lives before dosing of study drug until the end-of-study, for any medication ingested (whichever was longer), subjects were not allowed to use any kind of prescribed or over-the-counter medication, herbal remedy, vitamins, or minerals.

Dose Selection

The Food and Drug Administration guidance for industry Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers (2005) was used to determine the maximum recommended starting dose using the 80 mg/kg no observable adverse effect level established in cynomolgus monkeys to find a maximum recommended starting dose of 2.58 mg/kg. In the non-clinical pharmacology studies in cynomolgus monkeys, the minimum

effective dose was considered to be 2 mg/kg. Therefore, the starting dose in healthy volunteers had to be below this dose.

For clinical application, dose levels were transferred into fixed doses. Indeed, a variability of the numbers of platelets in blood is observed in healthy humans (between 250 000 to 450 000 per μL of blood), independently of the weight. It was, therefore, anticipated that the variation of weight would not influence the efficacy of the treatment. Also, a fixed dose seemed to be more appropriate for the infusion preparation of an emergency treatment like ACT017.

The targeted dose of 8 mg/kg from the nonclinical pharmacology program was converted into a targeted dose of 8 mg/kg in humans, resulting into a fixed dose of 500 mg for an adult (given an average weight of 60 kg). The maximum recommended starting dose and minimum effective dose described above converted into fixed doses according to the same principle were of around 155 and 120 mg, respectively. To initiate the trial, a starting dose lower than the minimum effective dose was selected.

Based on these calculations, single ascending doses of 62.5, 125, 250, 500, 1000, and 2000 mg ACT017 or matching placebo were administered (6 subjects on active compound and 2 subjects on placebo for each group)

Infusion Paradigm

The desired duration of effect for ACT017 was set to be longer than 6 hours in patients to cover the acute phase of ischemic stroke. Several infusion rates (8 mg/kg in 15 minutes, 1 hour and 6 hours) were tested in the cynomolgus monkey to determine the most appropriate rate to achieve complete inhibition of collagen-induced platelet aggregation after 6 hours (Figure 1 in the [online-only Data Supplement](#)). The results supported the use of a 6-hour intravenous infusion of ACT017 at 8 mg/kg through 2 successive phases. A loading dose, consisting of $\frac{1}{4}$ of the total dose, was administered within 15 minutes. The rest of the dose (maintenance dose), being $\frac{3}{4}$ of the total dose was administered within the remaining 5 hours and 45 minutes. Such administration in the nonclinical studies resulted in a profound inhibition of collagen-induced platelet aggregation starting rapidly, within 30 minutes of the beginning of the infusion, and lasting for at least 9 hours. A total infusion duration of 6 hours in this first-in-human study was considered sufficient to fulfill the objectives of the study.

Study Assessments

Safety was assessed by monitoring adverse events (AEs), vital signs, clinical examination, and safety laboratory results, including coagulation parameters. The infusion site was inspected frequently for local reactions. The Surgicutt BT procedure was performed predose and at selected time points throughout the study (15 minutes [end of the loading dose]; 6 hours [end of the infusion]; 14 hours, and 24 hours).

Serial blood samples for the pharmacokinetics analysis were collected predose and 10 time points over a period of 48 hours after the start of ACT017 infusion, processed to obtain platelet-free plasma, and stored at -80°C until analysis. In addition, urine was collected over selected intervals up to 24 hours postdose. A hybrid liquid chromatography-tandem mass spectrometry assay was developed for the determination of ACT017 concentration in plasma and urine. Extraction from plasma and urine samples was achieved with an initial affinity purification step, followed by solid phase extraction. The method allowed to measure free ACT017, but not platelet-bound ACT017. The method was validated in accordance with the current guidelines. Good precision, accuracy, reproducibility, and linear regression were obtained in a broad dynamic range, lower and higher limits of quantification for ACT017 being 0.300 and 400 $\mu\text{g}/\text{mL}$, respectively. Noncompartmental and compartmental pharmacokinetic analysis were performed.

The pharmacodynamics assessments for ACT017 activity were performed predose and at multiple time points during and after infusion by measuring collagen-induced platelet aggregation by light transmission aggregometry. Platelet aggregation was triggered by adding 2.5 $\mu\text{g}/\text{mL}$ collagen (Horm collagen, Nycomed, DE) to platelet-rich plasma obtained by centrifugation of citrated blood. Aggregation intensity at 250 s was used as pharmacodynamics parameter.

The level of GPVI expression at the platelet surface was assessed by flow cytometry using phycoerythrin-coupled 1G5 (Biocytex, France), a monoclonal antibody that binds to a different epitope than the one recognized by ACT017. Blood samples collected onto citrate, predose, at 6 hours and 24 hours after the start of the infusion were analyzed. Furthermore, the possibility for GPVI to be shed upon treatment was assessed by quantifying sGPVI (soluble GPVI) in plasma collected at different time, predose, and throughout the study, using a previously described method.¹⁹

An immunologic response was assessed by measuring antidrug antibodies (ADA) in serum samples obtained predose and at the follow-up visit using a validated method. The anti-ACT017 antibody assay runs using a Meso Scale Discovery Electrochemiluminescence platform. The samples were incubated with biotinylated ACT017 and SULFO-tag labeled ACT017 to allow the formation of a complex with antibodies against ACT017. Complexes were captured by biotinylated ACT017 on a standard Streptavidin Gold Multi-array 96 well plate and detection made on SULFO-tag labeled ACT017. Detection was performed in a Sector Imager S 600 (Mesoscale, Rockville, MD).

Statistical Evaluation

This was an exploratory study, and results are presented descriptively. No formal sample size calculation was performed. However, the design with 8 subjects per treatment group was considered sufficient to fulfill the objectives of this study. After completion, the database was locked. The demographic and baseline characteristics of volunteers are expressed as mean \pm SD. All other data are given as means \pm SD. Regarding pharmacokinetic parameters, the area under the curve and maximal ACT017 plasma concentrations were plotted as a function of the dose administered (linear regression) using Graph Pad Prism 7 software. Two-way ANOVA was used to compare means between multiple groups followed by multiple comparison test and performed using Prism7 software (GraphPad). Tolerability and safety data were listed and summarized descriptively.

Results

Healthy female (16, 33.33%) and male (32, 66.67%) subjects aged 22 to 65 years (median 56) were included between October 30, 2017 and January 16, 2018. Demographic and baseline characteristics are provided in Table 1. A flow diagram of the progress through this phase 1 trial is summarized in Figure 1.

All planned doses were administered as per protocol, and no dose adaptations were made. All subjects completed the study as per protocol. Subjects in the placebo group and in all treated cohorts had similar characteristics. Specifically, there were no differences in baseline BT, collagen-induced platelet aggregation, or platelet counts.

Safety and Tolerability

There were no deaths and no serious AEs, and none of the subjects discontinued the study because of a treatment-emergent AE. Overall, 17 subjects (35.42%) reported a total of 23 AEs. Of these subjects, 8 subjects (47.06%) reported at least 1 treatment-emergent AE possibly related to the study drug. The most frequent AEs were headache (8.33%) and head discomfort (6.25%). All AEs were of mild or moderate intensity and resolved during the study. None of the treatment-emergent AEs considered as related to the study drug were identified as bleeding related. An overview of all AEs is provided in Table 1 in the [online-only Data supplement](#).

Inspection of the infusion sites did not reveal any local reaction. There were no clinically significant findings or dose- and time-dependent effects on the laboratory safety

Table 1. Summary (Means and SD) of Demographic and Baseline Characteristics

	Placebo (n=12)	62.5 mg (n=6)	125 mg (n=6)	250 mg (n=6)	500 mg (n=6)	1000 mg (n=6)	2000 mg (n=6)
Study completed (n)	12	6	6	6	6	6	6
Age, y							
Mean (SD)	51.0 (15.93)	39.7 (15.92)	58.5 (4.76)	54.8 (9.39)	48.8 (16.24)	47.8 (15.08)	56.5 (7.18)
Sex							
Female, n (%)	3 (25)	0 (0)	4 (66.7)	2 (33.3)	2 (33.3)	2 (33.3)	3 (50)
Male, n (%)	9 (75)	6 (100)	2 (33.3)	4 (66.7)	4 (66.7)	4 (66.7)	3 (50)
Weight, kg							
Mean (SD)	78.32 (14.372)	87.85 (6.913)	73.02 (10.033)	74.20 (15.367)	73.42 (12.723)	73.60 (6.887)	79.85 (21.027)
BMI, kg/m ²							
Mean (SD)	24.40 (3.389)	26.98 (2.090)	25.07 (3.539)	24.47 (2.604)	23.13 (3.322)	22.60 (0.800)	24.93 (2.946)
Bleeding time, min							
Mean (SD)	5.79 (1.959)	5.58 (1.960)	6.08 (2.060)	5.83 (0.753)	5.08 (1.800)	4.50 (1.095)	5.00 (1.265)
Collagen-induced platelet aggregation							
Mean (SD)	79.342 (9.2751)	76.158 (10.2289)	80.178 (3.7071)	80.373 (2.6121)	80.837 (6.6319)	79.763 (8.0922)	83.275 (6.0907)
Platelet count (10 ⁹ /L)							
Mean (SD)	217.5 (35.96)	232.0 (38.47)	262.7 (55.93)	217.5 (43.02)	228.7 (35.52)	224.7 (42.27)	225.2 (34.14)

BMI indicates body mass index.

values. More specifically, there were no changes in hematologic parameters in particular red blood cells and leucocytes counts, hemoglobin levels, or any of the coagulation parameters (prothrombin time, activated partial thromboplastin time). Furthermore, there were no clinically significant changes in vital signs, ECGs, or physical examinations.

Bleeding Time

BT at screening ranged from 3 to 9.5 minutes (mean, 5.45; SD, 1.65; n=48). The BT variations observed at the different time points after initiation of the treatment are presented in Figure 2. Subjects that received the placebo had no deviation of the BT which was in the same range as at screening (3 to 9.5 minutes; mean, 5.36; SD, 1.58; n=60). In treated subjects, BT values ranged from 2.5 to 14 minutes (mean, 6.22; SD, 2.3; n=115; Figure IIA in the [online-only Data Supplement](#)). Occasionally, BT values out of the normal range were observed in some subjects, but there was no relation with the dose of ACT017. For example, one subject in the 125 mg group had an initial BT of 9 minutes, prolonged

to 12 minutes at 6 hours, decreased to 5.5 minutes at 14 hours, and increased again to 14 minutes at 24 hours; another in the group of 500 mg had an initial BT to 5 minutes that increased to 13.5 minutes at 15 minutes, 10 minutes at 6 and 14 hours, respectively, and 6.5 minutes at 24 hours. Comparison of the means±SD values did not show significant differences between the groups to the exception of the time point 6 hours in the 250 mg group (*P*=0.0248 versus placebo and *P*=0.0475 versus 62.5 mg). No clinically significant changes between postdose and predose BT values were noted at any of the ACT017 doses.

Pharmacokinetic Profile of ACT017

The results from the noncompartmental analysis are summarized in Table 2. As expected, the plasma concentration peaked at the end of the loading dose infusion at 15 minutes (Figure 3A). It slightly decreased thereafter until 1 hour when the steady state plasma concentration was achieved, and that lasted until the end of the infusion. After the end of infusion, a 2-step elimination profile, with a rapid and a

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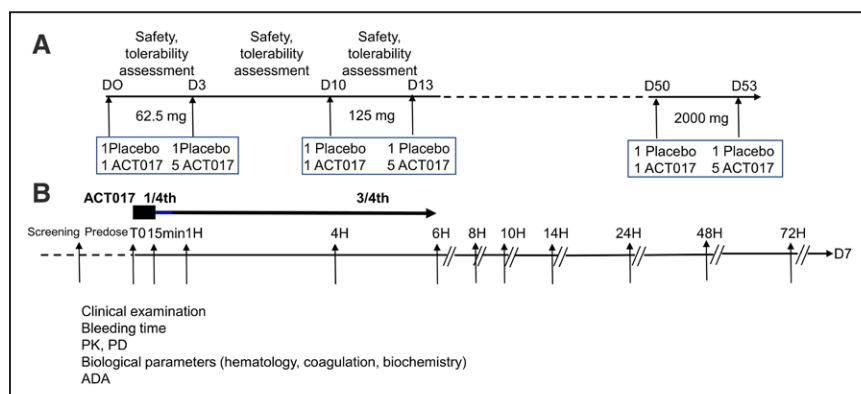


Figure 1. Study design. Flow chart **A** describes the mode of ACT017 administration. Flow chart **B** describes the time course of infusion and scheduled examinations. ADA indicates anti-drug antibody; PD, pharmacodynamics; and PK, pharmacokinetics.

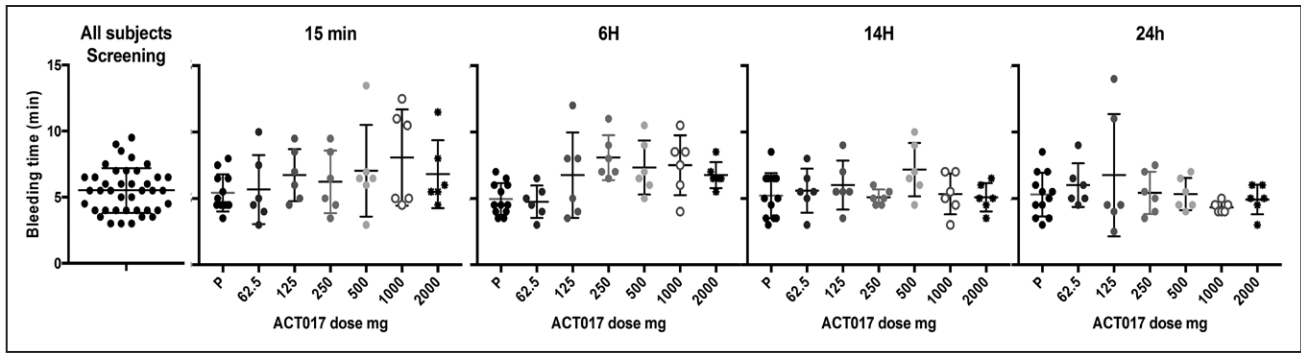


Figure 2. Bleeding time. The template bleeding time was measured at the screening, at the end of the loading and the infusion times and 14 and 24 h after the beginning of ACT017 administration. Individual values are shown for each dose and each time point with mean±SD.

slow phase, was observed that was in agreement with results obtained in cynomolgus monkeys. The area under the curve of the mean plasma concentration (AUC_{0-t}) as well as the maximal plasma concentrations (C_{max}) were proportional to the dose infused (Figure 3B and 3C) indicating that the pharmacokinetics of ACT017 is linear across the tested dose range of 62.5 to 2000 mg.

A compartmental pharmacokinetics analysis demonstrated that ACT017 had limited volumes of distribution of 4.1 and 7.3 L in plasma and the peripheral tissues, indicating that distribution was largely confined to the extracellular body water. The total body clearance was constant across the investigated dose range (with a mean of 2.65 L/h and ranging from 1.78 to 4.72 L/h between different individuals). Terminal half-life was 10.2 hours. Between subject variability of the pharmacokinetics parameters was small and was almost fully explained by differences in body weight. Pharmacokinetics simulations showed that the effect of mild and moderate renal impairment does not have large impact on pharmacokinetics profiles.

Urinary excretion of ACT017 has been measured in urines collected from time 0 to 6, 6 to 14, and 14 to 24 hours after the start of the infusion. The recovery of ACT017 in the urine was negligible for the 62.5, 125, and 250 mg doses and increased to 0.5 %, 6.5%, and 19.8%, respectively, for the 500, 1000, and 2000 mg doses. The majority of ACT017 was excreted during the first 6 hours, whereas hardly any ACT017 was detectable in the 14- to 24-hour urine collection period.

Effect of ACT017 on Platelet Aggregation

Collagen-induced platelet aggregation was measured before treatment and at different time points during and after the infusion of ACT017. The end point to quantify ACT017 biological efficacy was the intensity of aggregation at 250 s after the addition of collagen. Increasing doses of ACT017 impacted both the extent of the aggregation and the duration of the inhibitory effect (Figure 4 and Table II in the [online-only Data Supplement](#)). Variability between individuals was observed at low and intermediate doses. The lowest dose of 62.5 mg had a transient and subpharmacological effect limited to a 30%

Table 2. Pharmacokinetic Noncompartmental Analysis: Summary (Mean and SD) of ACT017 Plasma Pharmacokinetic Parameters Following a 6-Hour Intravenous Infusion of 62.5, 125, 250, 500, 1000, and 2000 mg of ACT017 to Healthy Adult Subjects

Group	A			B			C			D			E			F	
	62.5			125			250			500			1000			2000	
Parameters	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean±SD
C_{max} , µg/mL	6	2.89	0.485	6	6.62	1.40	6	14.5	3.31	6	26.1	3.70	6	59.9	18.9	6	109±25.7
T_{max} , h*	6	0.25 (0.25–1.00)		6	0.25 (0.25–0.30)		6	0.27 (0.25–0.28)		6	0.25 (0.25–6.00)		6	0.25 (0.25–0.35)		6	0.25 (0.12–0.25)
AUC_{0-t} , h/(µg·mL)	6	15.3	3.44	6	43.5	8.89	6	75.8	20.2	6	182	74.7	6	363	72.2	6	714±242
AUC_{0-inf} , h/(µg·mL)	1†	22.5	NA	6	45.1	9.64	4‡	75.0	19.3	6	189	76.6	6	373	72.1	6	726±245
λ_{z1} , 1/h	1†	0.507	NA	6	0.289	0.181	4‡	0.232	0.0258	6	0.107	0.0561	6	0.0620	0.0266	6	0.0550±0.00648
$t_{1/2}$, h	1†	1.37	NA	6	3.30	2.04	4‡	3.01	0.373	6	8.43	4.75	6	12.8	4.76	6	12.7±1.40
CL, L/h	1†	2.78	NA	6	2.87	0.578	4‡	3.48	0.787	6	3.03	1.17	6	2.77	0.553	6	3.06±1.09
Vz, L	1†	5.49	NA	6	12.5	5.72	4‡	15.1	3.75	6	31.1	8.13	6	49.5	18.0	6	57.1±23.4

N=no. of subjects for each treatment group. AUC indicates area under the curve; CL, total body clearance; NA, not applicable; and Vz, apparent volume of distribution.
 *Median (range).
 † AUC_{0-inf} , λ_{z1} , $t_{1/2}$, CL and Vz could not be estimated for 5 subjects and 1 subject in treatment group A and treatment group C, respectively.
 ‡ AUC_{0-inf} , λ_{z1} , $t_{1/2}$, CL and Vz were not reportable and were excluded from the statistical analysis for 1 subject in treatment group C since the subject did not meet the criteria (% AUC_{extrap} <20% and adjusted R^2 ≥0.80).

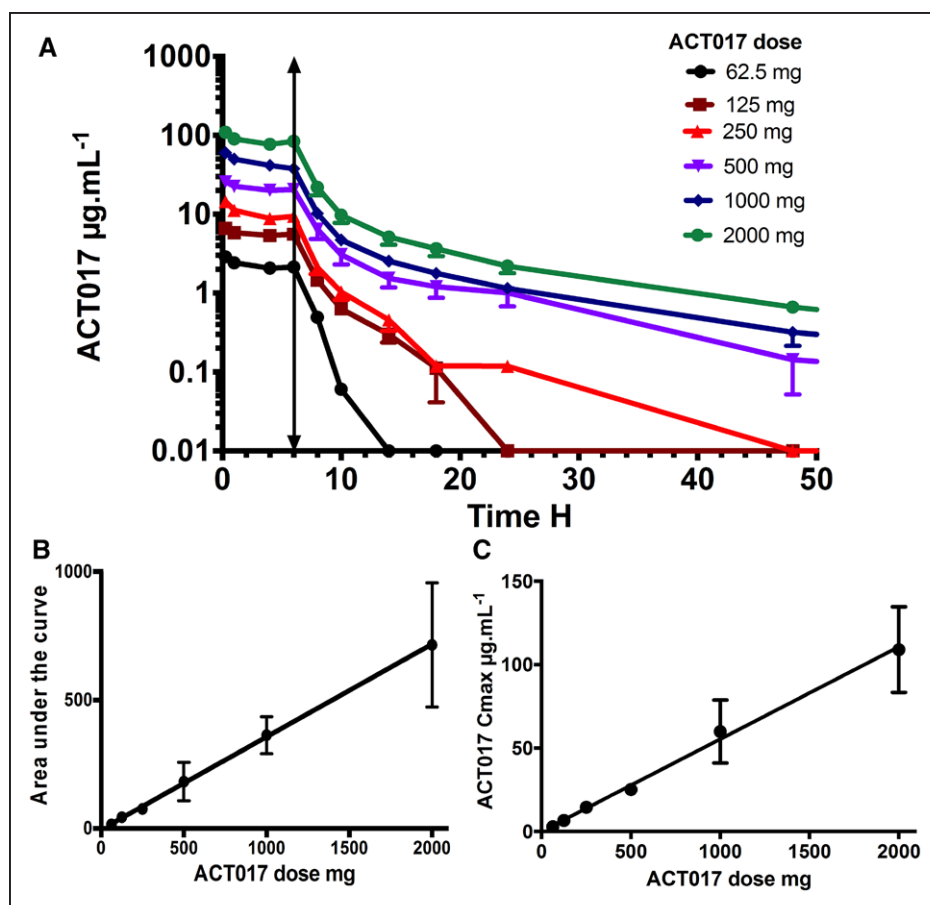


Figure 3. ACT017 pharmacokinetics in healthy subjects. **A**, The plasma concentration of ACT017 was measured at different time points during and after the administration of fixed doses of ACT017 as a 6 h infusion with one-fourth of the dose administered in the first 15 min. Results are the mean \pm SD of 6 subjects in each group. The black arrow indicates the end of the infusion. **B**, The area under the curve of the mean plasma concentration (AUC_{0-50}) is plotted as a function of the dose administered ($r^2 = 0.9994$; $P < 0.0001$). **C**, The maximal ACT017 concentration (C_{max}) is plotted as a function of the dose administered. ($r^2 = 0.9963$; $P < 0.0001$).

inhibition at the end of the loading dose and a complete reversal already at 1 hour after the start of the infusion. From the dose of 125 mg onwards, a plateau was observed starting at 1 hour after the beginning of the treatment. Its extent and the duration of this effect increased with the dose. Thus, during the length of the infusion, the aggregation intensity was reduced by about 60%, 80%, 80%, 90%, and 90% for the doses of 125, 250, 500, 1000, and 2000 mg, respectively. However, this maximal inhibitory effect lasted in total 6 hours for the 125 and 250 mg doses, with a prolongation to 8 hours at 500 mg and up to 18 hours at 1000 and 2000 mg. The recovery was more rapid at 1000 than 2000 mg with the inhibition being, respectively, of 55% and 80% at 24 hours and of 30% at 48 hours for 2000 mg only. Collagen-induced platelet aggregation was restored by >80% at time 48 hours in all subjects except 1 subject out of 6 in the 500 mg group and 4 out of 6 in the 2000 mg group. For the subjects of these 2 groups, the assay was repeated at day 7 and results showed that full recovery was obtained by that day.

Platelet Counts

Platelets counts were assessed 3 times before treatment and 10 times between 15 minutes after initiation of the infusion until day 3. (Figure 5A and Figure IIB in the [online-only Data](#)

[Supplement](#)). No significant variation in the platelet counts of the subjects treated with ACT017 compared with those who received placebo were observed.

GPVI Expression

Cell surface expression of GPVI was not altered in ACT017-treated subjects at any dose of ACT017 (Figure 5B). In addition, no significant variation in the plasma concentration of soluble GPVI of subjects treated with ACT017 was observed throughout the study and when compared with those who received placebo (Figure III in the [online-only Data Supplement](#)).

Antidrug Antibodies

Testing for ADA was performed in the serum from ACT017-treated subjects before treatment and at day 7 (Figure IV in the [online-only Data Supplement](#)). ADA were found negative in 32 subjects throughout the study. In 4 subjects, the signal was above the cutoff point before the treatment. This is not unusual because of the supersensitivity of ADA assay.²⁰ There was no change observed at the end of the study. These subjects did not present any significant difference as compared to the others in terms of safety and clinical parameters, as well as pharmacokinetics and pharmacodynamics.

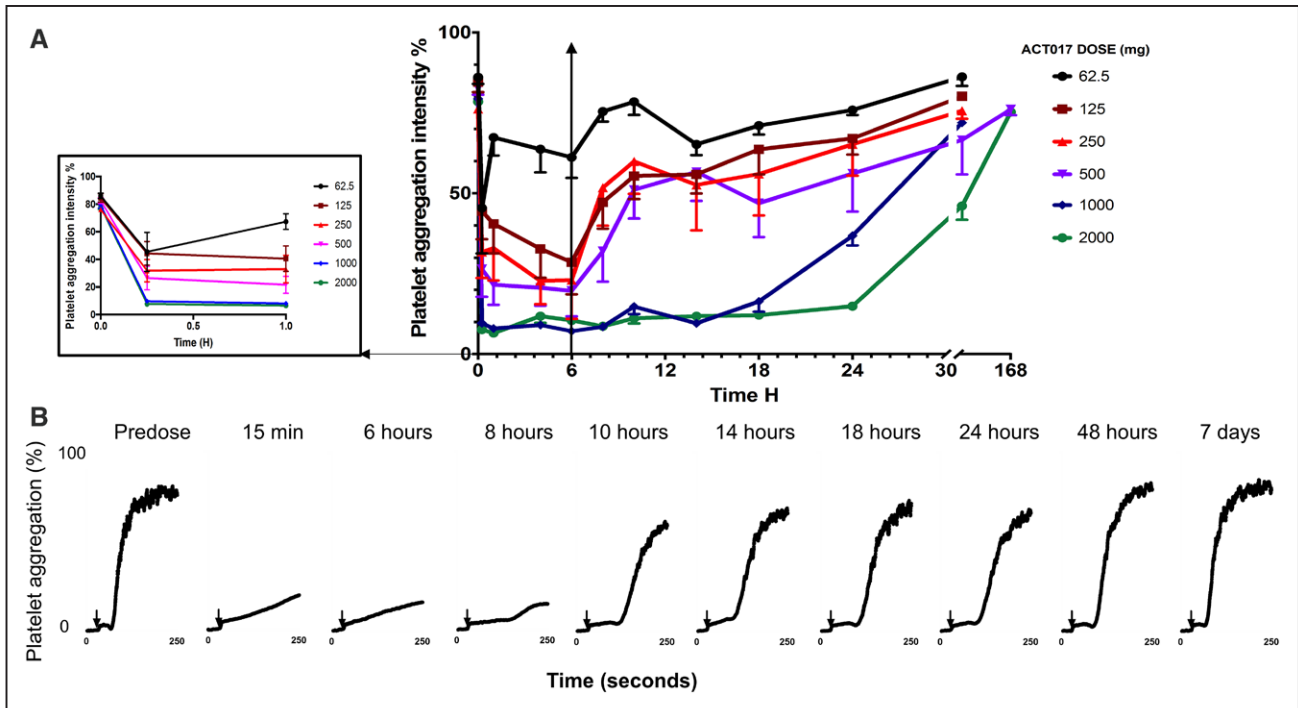


Figure 4. Inhibition of collagen-induced platelet aggregation. Collagen-induced platelet aggregation was measured before and at different time points during and after the administration of ACT017 at fixed doses. **A**, Aggregation intensity (mean±SD n=6 for each dose). The black arrow indicates the end of the infusion. An enlargement of the 0 to 1 h period is shown. **B**, Representative aggregation curves from 1 subject in the 1000 mg dose group. Arrows indicate the addition of collagen.

Discussion

In this first-in-human trial, the primary objective of the study was to evaluate the safety and tolerability of single ascending doses of ACT017. Secondary objectives were the evaluation of the pharmacokinetic and pharmacodynamic parameters. Infusions of up to 2000 mg of ACT017 were well tolerated, and no serious or severe AEs occurred. Importantly, although inhibition of platelet aggregation usually increases the risk of bleeding, no bleeding events and no prolongation of the BT was reported during the study period in agreement with the fact that GPVI is not strictly required for physiological hemostasis. The *in silico* evaluation of ACT017 immunogenicity indicated ACT017 falls within the range of licensed, nonimmunogenic antibodies.¹⁸ For the sampling for ADAs, the treatment duration, onset of ADA response, interference of ACT017 with the ADA assay and the presence of the ADA response, and the half-life of ADAs have to be considered. Interference of ACT017 with the ADA assay was not an issue because, for the highest dose of 2000 mg, there was practically no drug in plasma by day 7. In the present study, we got evidence that intravenous injection of ACT017 did not induce an early IgM response. We used the Meso Scale Discovery assay with a sensitivity exceeding the one imposed by the guidelines and samples taken 7 days after the exposure; these conditions being considered optimal by the Food and Drug Administration (Food and Drug Administration–Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products 2016). In future studies, sampling for ADA will be at baseline/screening (predose) and at 1 and 3 months which should match the onset and persistence kinetics of the IgM and IgG ADA responses.

The C_{max} of ACT017, as well as the mean ACT017 exposure area under the curve, were dose proportional over the dose range of 62.5 to 2000 mg. The plasma concentrations in the 62.5 and 125 mg dose groups were below the lower limit of quantification before the terminal elimination phase had been fully reached. At higher doses, the concentration-time profiles were biphasic indicating the distribution of ACT017 from plasma into peripheral tissue compartments.

Urinary excretion has previously been evidenced in pre-clinical studies in agreement with the molecular mass of the Fab (48 kDa). Here, urinary concentrations were below the lower limit of quantification for doses <500 mg and for all doses after the end of the infusion. Dilution in urine collected over rather a long period or degradation of ACT017 in urine over these periods may account for its low recovery.

ACT017 at the dose of 62.5 mg had little effect on collagen-induced platelet aggregation with only a partial and transient inhibition at the end of the loading dose infusion. The extent of the inhibition during the infusion period was dose-dependent for doses ranging from 125 to 500 mg and was maximal for higher doses (1000 and 2000 mg). The duration of the inhibition after the end of the infusion was also dose-dependent lasting >24 hours for the highest doses of 1000 and 2000 mg. During infusion, ACT017 was in high excess compared with GPVI. But because of the 6-hour duration of the infusion and the short half-life of ACT017, the plasma concentration at 12 hours is much lower, resulting in a displacement of the bound antibody all the more evidenced as the initial concentration was low. A high dose is thus required to maintain sufficient concentration over the longest time.

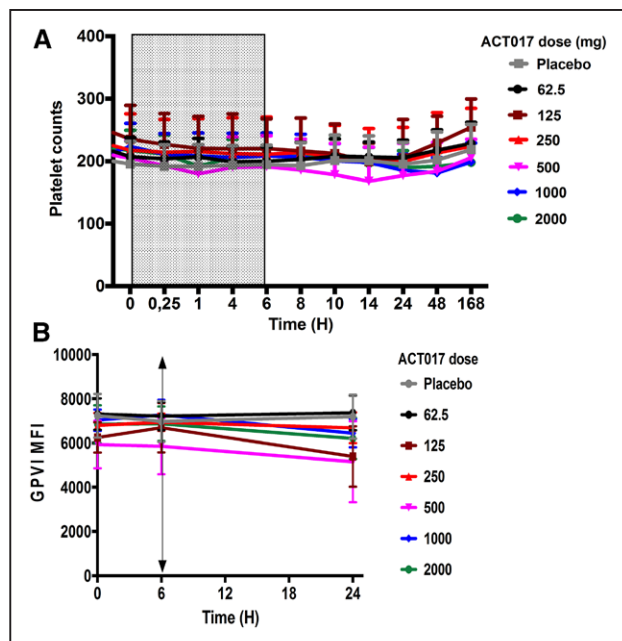


Figure 5. ACT017 administration does not impact the platelet count and GPVI (glycoprotein VI) expression. **A**, Platelet counts were determined at different time points before, during and after ACT017 administration. The period of ACT017 infusion is indicated by the shadowed area. Mean values \pm SD ($n=6$ for each ACT017 dose and 12 for the placebo). **B**, GPVI expression at the platelet surface was measured by flow cytometry before, at the end of the infusion and at 24 h. Results are expressed as mean platelet fluorescence (MFI) and are the mean values \pm SD ($n=6$ for each ACT017 dose and 12 for the placebo). The black arrow indicates the end of the infusion.

Importantly, the prolonged inhibition of collagen-induced platelet aggregation at these doses was not because of a modification of GPVI expression as ACT017 induced neither the internalization nor the shedding of GPVI as indicated by the stable level of GPVI expression at the platelet surface at all ACT017 doses and throughout the study and the absence of elevation of plasma sGPVI. This is in agreement with the results of preclinical studies in genetically modified mice expressing human GPVI¹⁷ and in cynomolgus monkeys.^{16,18} In fact, as platelet aggregation was triggered by collagen, integrin $\alpha 2\beta 1$ and amplification mechanisms (ADP release and thromboxane A2 synthesis) could counteract the inhibitory effect of ACT017.^{21–23} Indeed, subaggregating concentrations of ADP or U46619 (a thromboxane A2 analog restored, at least in part, the aggregation response induced by collagen in ACT017-loaded platelet-rich plasma (Figure VA in the [online-only Data Supplement](#)). The entry and the storage of ACT017 into the open canalicular system can also modulate the rate of the inhibition and its duration.²⁴

Regarding the specificity of ACT017, a limit of this phase 1 study was it did not allow to perform extensive platelet aggregation studies. However, studies conducted with Fab9O12, the predecessor of ACT017 demonstrated its specificity in mice humanized for GPVI¹⁷ and cynomolgus monkeys.¹⁶ Additionally, during the ACT017 pharmacological studies in cynomolgus monkeys, ADP-induced platelet aggregation was measured at different time points after a 6 hours infusion of the 8 mg/kg dose. No modification was observed (Figure IE in the [online-only Data Supplement](#)). Additionally, loading human platelet-rich plasma with ACT017 at the maximal plasma concentration reached

during the study ($100 \mu\text{g}\cdot\text{mL}^{-1}$) and a lower concentration ($25 \mu\text{g}\cdot\text{mL}^{-1}$) had no impact on ADP-, thrombin receptor agonist peptide- or ristocetin-induced platelet aggregation (Figure VB in the [online-only Data Supplement](#)) confirming the specificity of ACT017.

Mandatory data on safety, pharmacokinetics, and pharmacodynamics of ACT017 in healthy volunteers open the way to a novel therapeutic approach with an antiplatelet principle devoid of bleeding risk. However, it is important to be aware that the target population, that is, patients with acute ischemic stroke, is highly heterogeneous with respect to the mechanisms of thrombosis, and differences in safety, pharmacokinetics, and pharmacodynamics may exist. It is duly appreciated that bleeding may be higher, in particular, as other medications like r-tPA represent a standard-of-care at the acute phase of confirmed ischemic stroke, in the absence of a specific contraindication. A significant number of safeguard measures will be foreseen in an attempt to detect, monitor, handle, and mitigate such bleeding risk including brain imaging.

Overall, ACT017 had a good safety profile and predictable and consistent pharmacokinetics and pharmacodynamics in healthy volunteers. These results are the basis of subsequent assessment of ACT017 in safety and efficacy studies in the target population of patients with stroke.

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Authors' Contributions: C. Voors-Pette, P. Dogterom, and K. Lebozec performed the study, analyzed the data, and wrote the article; L. Jullien wrote the IMPD (Investigational Medicinal Product Dossier) and was in charge of the regulatory control; P. Billiald contributed to the design of ACT017 and the analysis of the data; P. Ferlan performed in vitro experiments; O. Favre-Bulle supervised the production of the clinical batch; L. Renaud and M. Machacek performed compartmentalized pharmacological analysis and contributed to the article; G. Avenard ordered and designed the study; P. Dogterom and Y. Plétan analyzed and interpreted the data and contributed to the article; M. Jandrot-Perrus designed the study, analyzed and interpreted the data, and wrote the article.

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Disclosures

C. Voors-Pette and P. Dogterom are employed by QPS Holdings LLC; L. Renaud and M. Machacek are employed by LYO-X GmbH; G. Avenard founded and is CEO, O. Favre-Bulle (COO) and Y. Plétan (CMO) are consultant, P. Billiald and M. Jandrot-Perrus founded and are consultant and K. Lebozec, L. Jullien, and P. Ferlan are employed by Acticor-Biotech.

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Highlights

- Inhibiting platelet GPVI (glycoprotein VI) interaction with its ligands is a promising strategy to develop new antiplatelet agents with a reduced bleeding risk.
- ACT017 is a first in class, humanized antibody fragment, selective and reversible GPVI antagonist.
- Administration of ACT017 in healthy subjects results in the inhibition of collagen-induced platelet aggregation the intensity and duration of which increases with the dose.
- Administration of ACT017 is well tolerated and does not impact the bleeding time.
- Our results suggest that GPVI antagonism with ACT017 is a new opportunity as a novel antiplatelet strategy.