Sponsor: Sanofi **Drug substance:** Caplacizumab	**Study Identifiers:** NCT03172208 **Study code:** ALX0681-C103
Title of the study: A Phase I Single Center, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Caplacizumab in Japanese and White Healthy Volunteers	
Study center: Subjects were enrolled in 1 study center in the United States.	
Study period: Date first participant enrolled: 05/Jun/2017 Date last participant completed: 19/Oct/2017 Study Status: Completed	
Phase of development: Phase I	
Objectives: Primary Objective: ● To assess the safety and tolerability of single ascending intravenous (i.v.) doses and a single subcutaneous (s.c.) dose of caplacizumab (Part I), and multiple s.c. doses of caplacizumab (Part II) in Japanese healthy subjects. Secondary Objectives: ● To compare the pharmacokinetics (PK) and pharmacodynamics (PD) profiles (total von Willebrand factor antigen [vWF:Ag] concentration levels, FVIII clotting activity [FVIII:C] and ristocetin-induced cofactor activity [RICO]) after single i.v. or s.c. administration of caplacizumab in Japanese and White healthy subjects. ● To evaluate the immunogenicity of caplacizumab (anti-drug antibodies [ADA]) in Japanese healthy subjects.	

Methodology:

This was a Phase I, single-center, randomized, double-blind, placebo-controlled, 2-part study to evaluate the safety, tolerability, PK/PD profile and immunogenicity of single i.v. and s.c. doses (Part I) or multiple s.c. doses (Part II) of caplacizumab in Japanese and White healthy volunteers.

Each part of the study was performed in a different set of subjects. Subjects not fulfilling the criteria for inclusion in the PK population had to be replaced.

Part I (SINGLE DOSE):

- Cohort 1/Group 1: 8 Japanese subjects were to be randomized 3:1 to receive a 5 mg i.v. dose of caplacizumab or placebo
- Cohort 2/Group 2: 10 Japanese subjects and 10 age- and body mass index (BMI)-matched White subjects were to be randomized 4:1 to receive a 10 mg i.v. dose of caplacizumab or placebo
- Cohort 2/Group 3: 10 Japanese subjects and 10 age- and BMI-matched White subjects were to be randomized 4:1 to receive a 10 mg s.c. dose of caplacizumab or placebo

This part of the study assessed the safety, tolerability, PK/PD profile and immunogenicity of caplacizumab after a single 5 mg i.v. administration in Japanese subjects and after single 10 mg i.v. and s.c. administrations in Japanese and White subjects.

Part II (MULTIPLE DOSES):

- Cohort 3/Group 4: 12 Japanese subjects were to be randomized 3:1 to receive a 10 mg s.c. dose of caplacizumab or placebo once daily for 7 days.

This part of the study assessed the safety, tolerability, PK/PD profile and immunogenicity of caplacizumab in Japanese subjects after 7 days of daily 10 mg s.c. administrations.

PART I

Screening period: Consisted of a Screening Visit any day between Day -28 and Day -2 and a mandatory Screening Visit on Day -1.

Treatment period: Subjects in Part I of the study were required to stay at the study site until all assessments of Day 4 had been completed. After Day 4, ambulatory (i.e., outpatient) visits were planned on Day 5 and Day 6 at 96 and 120 hours postdose, respectively, and on Day 14 ± 1.

Follow-up (FU) period: 4 weeks after dose (FU Visit = Day 28 ± 3)

PART II

Screening period: Consisted of a Screening Visit any day between Day -28 and Day -2 and a mandatory Screening Visit on Day -1.

Treatment period: Subjects in Part II of the study were required to stay at the study site from Day -1 until all Day 2 assessments had been completed, and from Day 7 until all Day 8 assessments had been completed. Ambulatory visits were planned on the morning of Days 3, 4, 5, 6 (48, 72, 96 and 120 hours post first dose); on Days 9, 10, 11 and 12 (at 48, 72, 96 and 120 hours post last dose); and on Day 20 ± 1.

Follow-up period: 4 weeks after last dose (FU Visit = Day 34 ± 3)

Dose Escalation and Safety Review

Proceeding to the higher dose or multiple dosing only occurred after the completion of a Safety Monitoring Review (SMR). The review of data from subjects in Group 1 started once all subjects in Group 1 had reached Day 14 (SMR 1); the first study drug administration of Japanese subjects in Cohort 2 started upon a positive SMR 1 outcome. The review of data from Japanese subjects in Cohort 2 (SMR 2) started once all Japanese subjects in Cohort 2 had reached Day 14; the first study drug administration of subjects in Group 4 started upon a positive SMR 2 outcome.

Number of participants:

At least 60 healthy subjects (40 Japanese subjects and 20 White subjects) were planned to take part in this study and be divided into 3 cohorts / 4 groups as described in the Study Design section above.

A total of 60 subjects were enrolled and randomized as planned. Fifty-nine subjects completed the study; 1 Japanese subject who received 10 mg i.v. caplacizumab was lost to follow-up and withdrawn.

Diagnosis and criteria for inclusion:

Healthy male and female volunteers between the ages of ≥ 18 and 65 years who were Japanese or White.
The main criteria for inclusion were as follows:

- Healthy volunteers: subjects were able to comprehend and willing to sign an informed consent form (ICF) and with satisfactory medical assessment with no clinically significant or relevant abnormalities in medical history, vital signs, physical examination, electrocardiogram (ECG) and laboratory evaluation (hematology, biochemistry, urinalysis, coagulation parameters) as assessed by the Investigator
- Gender: male or female
- Between the ages of ≥ 18 and 65 years at the time of consent
- Body mass index (BMI) between ≥ 18 kg/m² and < 30 kg/m² at time of screening
- Body weight between ≥ 45 kg and < 100 kg (99 and < 220 lbs)
- Baseline vWF:Ag between ≥ 60% and < 170% (0.6-1.7 IU/mL)
- Female subjects of childbearing potential (excluding postmenopausal women [≥1 year since last menses], sterilized, ovariectomized and hysterectomized women) must have had negative pregnancy tests on the ambulatory Screening Visit (between Day -28 and Day -2) and on the Day -1 Screening Visit and must have agreed to use a generally accepted adequate contraceptive method from screening until at least 2 months after last dosing.
- Only Japanese or White subjects fulfilling the following descriptions:

- Japanese subjects must not have lived outside of Japan for over 10 years and must have had ethnic Japanese parents and grandparents born in Japan.
- White subjects must have had origins in any of the original people of Europe, the Middle East or North Africa and must have had White parents and grandparents.

Study products

Caplacizumab 10 mg – lyophilized powder for solution for injection
- Presented in ISO 2R glass vial with FluroTec® Butyl stopper filled with lyophilizate containing 12.5 mg caplacizumab and excipients (see below)
- Active substance: caplacizumab (anti-vWF Nanobody)
- Activity: caplacizumab is directed towards the A1 domain of vWF and specifically inhibits the interaction of (ultra-large multimers [UL])vWF with the platelet receptor GPIb-IX-V, thereby preventing (UL)vWF mediated platelet aggregation
- Strength: One vial contains 12.5 mg of caplacizumab Excipients: 0.21 mg citric acid anhydrous, 5.58 mg tri-sodium citrate dihydrate, 70 mg sucrose, 0.11 mg polysorbate-80 per vial (pH 6.5 ± 0.5)
- Dosage form: powder for solution for injection; reconstitution with WFI yields solution for injection
- Route of administration: i.v. or s.c.

Placebo - lyophilized powder for solution for injection:
- Presented in ISO 2R flass vial with FluroTec® Butyl stopper filled with placebo lyophilisate containing excipients only (see below)
- Active substance: not applicable, the composition of placebo is the same as that of active investigational medicinal product (IMP), without the active ingredient
- Activity, strength: not applicable
- Excipients: 0.21 mg citric acid anhydrous, 5.58 mg trisodium citrate dihydrate, 70 mg sucrose, 0.11 mg polysorbate-80 per vial (pH 6.5 ± 0.5)
- Dosage form: powder for solution for injection; reconstitution with WFI yields solution for injection
- Route of administration: i.v. or s.c.

Study duration:

In Part I, the study duration per subject was a maximum of 55 days.
In Part II, the study duration per subject was a maximum of 57 days.

Criteria for evaluation:

Pharmacokinetics:

The PK parameters determined by non-compartmental analysis for caplacizumab, based on actual sampling time in plasma and urine following since- and multiple-dose administration, were as shown below:

Plasma

Cmax	Value of the maximum plasma concentration
Tmax	Time corresponding to occurrence of Cmax
C_{last}	Last estimated quantifiable plasma concentration
$t_{½}$	Apparent terminal elimination half-life (Groups 1-3 only)
λ_z	Terminal elimination rate constant (Groups 1-3 only)
AUC_{0-t}	Area under the plasma concentration-time curve observed from time 0 hour up to the last measurable data point computed using the linear up/log down trapezoidal rule
t_{last}	Time of last measurable concentration (used for AUC_{0-t})
AUC_{0-24}	Area under the plasma concentration-time curve observed from time 0 hour up to the 24 hour data point computed using the linear up/log down trapezoidal rule (Groups 2-3 only)
$AUC_{0-\infty}$	Area under the curve extrapolated to infinity, calculated as: $AUC = AUC_{0-t} + C_{last} / \lambda_z$ (Groups 1-3 only)
AUC_{τ}	Area under the plasma concentration-time curve observed over a dosing interval computed using the linear up/log down trapezoidal rule
CL/(F)	(Apparent) total body clearance following intravenous (oral) administration (Groups 1-3 only)
Vz/(F)	(Apparent) volume of distribution during terminal phase following intravenous (oral) administration (Group 1-3 only)
F	Bioavailability estimated for the 10 mg dose from mean exposure parameters from subjects in the i.v. and s.c. Cohort 2
R	Accumulation index, calculated as AUC_{τ} Day 7/ AUC_{τ} Day 1 in subjects of Cohort 3

Urine

fe	Fraction of the administered drug excreted in urine
Ae	Cumulative amount of unchanged drug excreted in urine
CL_R	Renal clearance calculated as Ae / $AUC_{0-\infty}$ (Part I) or AUC_{τ} (Part II)
Cum $f_{e(tz)}$	Cumulative fraction of unchanged drug excreted in urine

Pharmacodynamics:

The following PD parameters were collected:

- Plasma concentrations of total vWF:Ag
- RICO (platelet and not antibody-based activity)
- FVIII:C (clotting activity)

Immunogenicity:

The immunogenicity assay strategy consisted of 2 assays, a conventional ADA assay potentially supplemented with analysis in the modified ADA (mADA) assay. Only subjects classified as “Pre Ab positive – equivocal (EQ)” in the ADA assay had their samples analyzed in the mADA assay.

Parameters provided were:

- Anti-drug antibody (ADA) log10(titer) per sample and subject classification per subject
- mADA log10(titer) per sample and subject classification per subject, only for a subset of subjects
- Overall subject classification (based on ADA and/or mADA assay results)

Safety:

The safety assessments were standard procedures for ensuring subject safety and well-being during the conduct of a clinical study. Safety and tolerability were assessed by the monitoring of adverse events (AEs), vital signs, ECGs and clinical laboratory test results.

Statistical methods:

Analysis populations:

The Safety Population included all subjects who received at least 1 dose of study drug. The PK Population included all subjects who received study drug and had sufficient and interpretable PK data. Subjects missing a dose during Part II, missing predose PK sample, missing more than 2 consecutive PK samples or having less than 80% of PK samples results available were considered non-eligible for the PK Population.

In case of a missed dose/wrong group assignment in Part II, subjects were excluded from the PK population.

Pharmacokinetics evaluation:
In Part II, trough levels were plotted over time to visually check the attainment of steady-state conditions.
In cases when any of the urine analyte was missing or if the volume was missing or incomplete, the amount of drug excreted in urine (Ae) and derived urinary PK parameters could not be estimated and were listed as “not calculated” (NC). Derived urinary parameters (Ae, fe, CLR, cumulative fe) were not calculated for these individuals.
In Groups 2 and 3 of Part I, analysis of variance (ANOVA) models were used to compare log-transformed values of caplacizumab AUC0-∞, AUC0-t, Cmax, along with 90% confidence interval (CI), using the data from the White subjects as reference.
All PK parameters were summarized by descriptive statistics, including arithmetic mean, standard deviation, median and range.
The race effect on treatment PK was assessed by constructing analysis of covariance (ANCOVA) models for i.v. (Group 2) and s.c. (Group 3) PK parameters, separately. The ANCOVA model had log-transformed AUC0-∞, AUC0-24 and Cmax as dependent variables and race, sex and body weight as explanatory variables.
Pharmacodynamics evaluation:
The PD parameters (vWF:Ag, FVIII:C, RICO) were explored graphically and summarized by descriptive statistics, including arithmetic mean, standard deviation, median and range.
The PK/PD (vWF:Ag) relationship was examined via a non-linear mixed-effects modelling approach, testing the effect of race on model parameters.
Immunogenicity evaluation:
Immunogenicity was assessed through listing of individual results by subject and summary tables. Immunogenicity data were correlated with PK and PD readout. In addition, immunogenicity was correlated with possible safety findings.
Safety evaluation:
The analysis of safety data was based on the Safety Population. Safety was assessed based on reported AEs, abnormal findings from physical examinations, vital signs measurements, 12-lead ECGs and clinical laboratory results. Adverse events and medications were coded using MedDRA Version 20.0 and the World Health Organization Drug Dictionary Enhanced, respectively. All AEs reported during the study were included in by-subject data listings and tabulated by MedDRA system organ class (SOC) and preferred term. Treatment-emergent AEs (TEAEs) were summarized overall, by relationship and by severity. Serious AEs and events resulting in study discontinuation are listed. Exposure to study drug and study drug compliance were tabulated. Summaries of ECG measurements were prepared by dose cohort using descriptive statistics. ECG data are also presented in a by-subject listing.
For each continuous laboratory parameter, results were categorized as low, normal or high, based on the laboratory normal ranges. Frequencies and percentages were presented by dose group and race for subjects who had a shift in category from baseline to any post-dosing assessment. All out-of-range and clinically significant laboratory results are identified in subject data listings. Actual values and changes from baseline over time for laboratory parameters were summarized descriptively by dose group and race.
Clinical laboratory biochemistry, hematology, coagulation parameters and urinalysis are listed. All out-of-range and clinically significant laboratory results are identified in subject data listings. Change from baseline values are included in the listings.
Actual values and changes from baseline over time for vital signs and 12-lead ECGs were summarized by dose group and race.
Abnormal findings in physical and gynecological examination are listed.

Summary Results:

Pharmacokinetics:

Part I of this study was designed to assess the PK of a single dose of caplacizumab after i.v. or s.c. administration to healthy Japanese and White subjects. Part II of this study was designed to assess the PK after multiple subcutaneous administration of caplacizumab to healthy Japanese subjects.

Single Intravenous Dosing:

Caplacizumab PK profile is determined by target-mediated drug disposition as only drug bound to the target vWF is retained in circulation. Consequently after i.v. administration at 5 and 10 mg, non-linear kinetics were observed.

Japanese and White subjects administered 10 mg of caplacizumab i.v. showed similar mean values for C_{max}, AUC_{0-24} and AUC_{0-inf} when the parameters were normalized by administered dose/body weight and baseline vWF:Ag. The GMR for the C_{max}, AUC_{0-24} and AUC_{0-inf} were 1.11, 1.028 and 1.072, respectively. The results for White subjects showed slightly higher variability. The Japanese and White subjects showed similar mean t1/2 values at approximately 20 hours.

Single Subcutaneous Dosing:

Japanese and White subjects administered 10 mg of caplacizumab s.c. showed similar mean values for C_{max}, AUC_{0-24} and AUC_{0-inf} when the parameters were normalized by administered dose/body weight and baseline vWF:Ag. The GMR for the C_{max}, AUC_{0-24} and AUC_{0-inf} were 0.9919, 1.053 and 0.9476, respectively. The Japanese and White subjects showed similar mean $t_{1/2}$ values at approximately 37 hours. The Japanese and White subjects had similar CL/F and Vz/F mean values.

The Japanese and White subjects had similar relative bioavailability. The F AUC_{0-inf} was 1.871 for the Japanese and 1.838 for the White subjects. Subcutaneous administration resulted in flip-flop kinetics where absorption/distribution rate was slower than the elimination rate.

While the study was far from statistically powered to demonstrate formal “bioequivalence”, the overall data indicate no important PK differences between Japanese and White subjects.

Thus, no PK-based dose-adjustment based on ethnicity appears warranted.

Multiple Subcutaneous Dosing:

In Part II, after 7 days of dosing the drug exposure decreased. The Day 1 AUC_{0-24} was 11360 (4273) h•ng/mL and the Day 7 AUC_{0-24} was 8439 (1836) h•ng/mL. This decrease on Day 7 was related to the modulation of the target and thus a decrease in the amount of the target vWF.

Pharmacodynamics:

During this study, subjects in Part I received a single 5 mg i.v. dose of caplacizumab or placebo, a single 10 mg i.v. dose of caplacizumab or placebo or a single 10 mg s.c. dose of caplacizumab or placebo; subjects in Part II received a daily s.c. dose of 10 mg s.c. caplacizumab or placebo for 7 days.

Single Intravenous Dosing:

For subjects receiving a single i.v. dose of IMP, suppression of RICO was comparable between Japanese and White subjects who received caplacizumab, with complete RICO activity suppression (< 20%) up to 24 hours. Recovery occurred between 72 through 96 hours for subjects dosed with 10 mg caplacizumab versus 48 hours for subjects dosed with 5 mg caplacizumab. No significant suppression was observed in the placebo groups.

The largest drop in vWF:Ag levels occurred in White subjects for the 10 mg i.v. caplacizumab dose group. Baseline values in this group were lower compared to other dose groups; the greatest mean (SD) change from baseline in White subjects for the 10 mg i.v. caplacizumab dose group was -37.43 (26.59), compared to a greatest mean (SD) change from baseline in Japanese subjects for this dose group of -18.34 (29.03). Recovery times to baseline levels in all caplacizumab dose groups were as follows: 24 hours for Japanese subjects dosed with 5 mg caplacizumab, 48 hours for Japanese subjects dosed with 10 mg caplacizumab and 48 hours for White subjects dosed with 10 mg caplacizumab. No significant decrease in vWF:Ag levels was observed in the placebo groups.

A small decrease in FVIII:C levels was observed in all White and Japanese subjects treated with caplacizumab. Recovery times to baseline levels in all caplacizumab dose groups were as follows: 24 hours for Japanese subjects dosed with 5 mg caplacizumab, 48 hours for Japanese and White subjects dosed with 10 mg caplacizumab. No significant drop in FVIII:C levels was observed in the placebo groups.

Single Subcutaneous Dosing:

Comparable inhibition of RICO activity was observed for White and Japanese subjects dosed with 10 mg s.c. caplacizumab up to 48 hours. Recovery to baseline was 96 hours for both Japanese and White subjects. No significant inhibition of RICO activity was observed in the placebo groups.

A similar decrease in vWF:Ag levels occurred in Japanese and White subjects for the 10 mg s.c. caplacizumab dose group. Comparable changes from baseline were also observed, with the maximum decrease occurring at 24 hours: mean changes (SD) from baseline were -49.88 (23.48) in Japanese subjects and -43.08 (14.74) in White subjects. Recovery to baseline levels was 72 hours for both Japanese and White subjects. There were no changes in placebo; vWF:Ag levels remained stable.

A small decrease in FVIII:C levels was observed in all caplacizumab treatment groups. The decrease was temporary, and a similar pattern was observed for Japanese and White subjects. Recovery to baseline was 72 hours for both Japanese and White subjects. No significant decrease was observed in the placebo groups.

Multiple Subcutaneous Dosing:

Daily s.c. administration of caplacizumab to Japanese subjects up to Day 7 (168 hours) resulted in complete RICO inhibition up to Day 8 (192 hours). Recovery to baseline occurred 96 hours after the last dose at Day 7, which was comparable to the recovery seen for single-dose s.c. administration. No significant inhibition was observed in the placebo group.

A decrease in vWF:Ag levels was observed in both caplacizumab and placebo dose groups.

At baseline, the mean (SD) vWF:Ag levels in the placebo group (81.27% [17.13]) were lower than those in the caplacizumab group (90.20% [29.87]). The maximum (SD) changes from baseline were observed at Day 7 predose (-57.41 [24.45] in caplacizumab and -22.87 [2.082] in placebo dose groups). The placebo group had lower baseline vWF:Ag levels (mean [SD] 81.27% [17.13] versus 98.20% [29.87] in the caplacizumab dose group), but the vWF:Ag levels remained rather constant throughout the treatment period.

Recovery to baseline levels was 72 hours after the last dose at Day 7, which was comparable to the recovery seen for single-dose s.c. administration.

A decrease in FVIII:C levels was observed in the caplacizumab dose group. The maximum (SD) changes from baseline were observed at Day 7 predose (-48.84 [19.84] in the caplacizumab dose group) and Day 5 (-10.80 [4.713] in the placebo group). Recovery to baseline levels was 72 hours after the last dose at Day 7, which was comparable to the recovery seen for single-dose s.c. administration. No significant decrease was observed in the placebo group.

Immunogenicity:

During this study, subjects in Part I received a single 5 mg i.v. dose of caplacizumab or placebo, a single 10 mg i.v. dose of caplacizumab or placebo or a single 10 mg s.c. dose of caplacizumab or placebo; subjects in Part II received a daily s.c. dose of 10 mg s.c. caplacizumab or placebo for 7 days.

Within the caplacizumab-treated subjects (either single-dose or multiple-dose and either i.v. or s.c.), prevalence of pre-Ab in White versus Japanese subjects was 12.5% (2/16) and 16.1% (5/31), respectively. Subjects were classified as “Pre-Ab positive – Equivocal”, with a prevalence of 8.5% (4/47) and 15.4% (2/13) in the caplacizumab-treated subjects and placebo-treated subjects, respectively.

When comparing over the different dose groups; pre-Ab prevalence in single i.v. dosing, single s.c. dosing and multiple s.c. dosing was 9.1% (2/22), 6.3% (1/16) and 44.4% (4/9), respectively. “Pre-Ab positive – Equivocal” classifications were 4.5% (1/22), 6.3% (1/16) and 22.2% (2/9) in single i.v. dosing, single s.c. dosing and multiple s.c. dosing, respectively. None of the pre-Ab positive or “Pre-Ab positive – Equivocal” subjects developed a TE ADA response. Overall, TE ADA were detected in none of the subjects (caplacizumab-treated or placebo-treated).

Safety:

No deaths, serious AEs (SAEs), TEAEs leading to study discontinuation or severe/serious hypersensitivity TEAEs occurred in the study.

Overall, 12 subjects experienced at least 1 IMP-related TEAE (Part I: 1 [16.7%] Japanese subject receiving 5 mg i.v. caplacizumab, 3 [37.5%] White subjects receiving 10 mg i.v. caplacizumab, 1 [12.5%] Japanese subject receiving 10 mg i.v. caplacizumab, 2 [25.0%] White subjects receiving 10 mg s.c. caplacizumab and 1 [16.7%] Japanese subject receiving placebo; Part II: 4 [44.4%] Japanese subjects receiving 10 mg s.c. caplacizumab daily). No TEAEs were reported by Japanese subjects receiving 10 mg s.c. caplacizumab (Part I), White subjects receiving placebo (Part I) or Japanese subjects receiving daily placebo (Part II).

With the exception of the 2 moderate TEAEs nausea and vomiting, which were each experienced by 1 (11.1%) Japanese subject receiving 10 mg s.c. calplacizumab daily (Part II), all TEAEs in the study were considered mild in severity. All TEAEs were considered resolved or resolving by the end of the study.

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