Sponsor: Sanofi **Drug substance:** Caplacizumab	**Study Identifiers:** NCT02189733; EudraCT: 2014-001294-13 **Study code:** ALX0681-C102 (QBR117354)
Title of the study: A Phase I, Single Center, Open-Label, Randomized, Single Dose Cross-Over Study in Healthy Male Subjects to Investigate the Bioequivalence and Tolerability of Liquid and Reconstituted Lyophilized Subcutaneous Formulations of Caplacizumab	
Study center: 1 center in UK	
Study period: Date first participant enrolled: 01/Jul/2014 Date last participant completed: 20/Sep/2014 Study Status: Completed	
Phase of development: I	
Objectives: The primary objective of the study was: ● To evaluate the pharmacokinetic (PK) characteristics and demonstrate bioequivalence of a reconstituted new lyophilized formulation of caplacizumab for subcutaneous (SC) injection (A: new formulation) as compared to an equal nominal SC dose of the reference liquid formulation of caplacizumab (B: reference formulation). The secondary objective of the study was: ● To compare the safety and tolerability, and the pharmacodynamic (PD) parameters of the new formulation with those of the reference formulation.	

Methodology:

This study was a Phase I, single center, open-label, randomized, single dose, 2-way 2-period cross-over study in healthy male subjects.

It was planned to randomize 24 subjects in a 1:1 ratio to either treatment sequence AB (12 subjects) or treatment sequence BA (12 subjects).

In Part 1 of the study, subjects received either:

Treatment A (new formulation; reconstituted lyophilized drug product): single dose of 10 mg (0.8 mL) reconstituted lyophilized solution (12.5 mg/mL) of anti-von Willebrand factor (vWF) Nanobody caplacizumab, administered by single SC injection.

Or

Treatment B (reference formulation; liquid formulation): single dose of 10 mg (2 × 1 mL) liquid formulation (5 mg/mL) of anti-vWF Nanobody caplacizumab, administered as 2 SC injections of 1 mL each.

In Part 2 of the study, subjects received the alternative treatment.

Subjects were screened to confirm eligibility for participation in the study between 28 and 7 days prior to dosing. Both treatment parts consisted of 14 days, including dosing on Day 1 (in Part 1; Day 15 for Part 2) and an assessment period (for Part 1 this corresponds to a wash-out period) until Day 14 (in Part 1; Day 28 for Part 2).

Eligible subjects were admitted to the study site on Day -1 (in Part 1; Day 14 in Part 2) and received a single dose of caplacizumab on Day 1 (in Part 1; Day 15 in Part 2).

Subjects were resident at the study site until the morning of Day 4 (in Part 1; Day 18 in Part 2) to allow adequate observation during the first 72 h after study drug administration. Subjects returned for 5 ambulatory planned visits 4, 5, 6, 7 and 10 days after each dose, and for 2 follow-up (FU) visits: FU visit 1, 2 weeks after the second dose (Day 29) and FU visit 2, 4 weeks after the second dose (Day 43).

Number of participants:

Planned: 24; Enrolled: 24; Randomized: 24; Completed: 24; Withdrawn: 0

Diagnosis and criteria for inclusion:

Male Caucasians (white European) aged 18 to 55 years, inclusive, with a body weight of 55 to 100 kg and a body mass index between 18.5 and 30.0 kg/m^2.

Test products

Treatment A (new test formulation; reconstituted lyophilized drug product:

A single dose of 10 mg (0.8 mL) reconstituted lyophilized solution (12.5 mg/mL) of anti-vWF Nanobody caplacizumab, administered by single SC injection in the abdominal region.

Treatment B (reference formulation; liquid formulation):

A single dose of 10 mg (2 × 1 mL) liquid formulation (5 mg/mL) of anti-vWF Nanobody caplacizumab, administered as 2 SC injections of 1 mL each in the abdominal region.

The second mL of caplacizumab was taken immediately after the first mL from the same vial.

Duration of treatment:

Single 10 mg doses of caplacizumab were administered by SC injection on 2 occasions separated by a minimum of 14 days. Subjects had 2 FU visits: FU visit 1, 2 weeks after the second dose (Day 29) and FU visit 2, 4 weeks after the second dose (Day 43).

Criteria for evaluation

Pharmacokinetics

The following PK parameters for concentrations of caplacizumab in plasma were estimated where possible for each subject and treatment unless otherwise specified:

C_{max} and t_{max}	Value and time of the maximum plasma concentration obtained from the observed concentration versus (vs) time curves.
C_{max}_D	C_{max} normalized to actual dose, computed as: C_{max}/Actual Dose.
C_{last}	Last estimated quantifiable concentration directly obtained from the observed concentration vs time curves.
$AUC_{0\text{-}last}$	Area under the plasma concentration vs time curve observed from time 0 h up to the last measurable data point computed using the linear up/log down trapezoidal rule.
$AUC_{0\text{-}last}$_D	$AUC_{0\text{-}last}$ dose normalized to actual dose, computed as: $AUC_{0\text{-}last}$/Actual Dose.
$AUC_{0\text{-}\infty}$	Area under the curve extrapolated to infinity, calculated as: $AUC_{0\text{-}last} + C_{last}/\lambda z$.
$AUC_{0\text{-}\infty}$_D	$AUC_{0\text{-}\infty}$ dose normalized to actual dose, computed as: $AUC_{0\text{-}\infty}$/Actual Dose.
$\%AUC_{extra}$	Relative area under the curve between the last measured time and infinity, calculated as: $100*(AUC_{0\text{-}\infty} - AUC_{0\text{-}last})/AUC_{0\text{-}\infty}$

F_{rel}	Relative bioavailability, calculated as: 100*($AUC_{0-\infty}$_D [Formulation A]/$AUC_{0-\infty}$_D [Formulation B])
λz	Terminal elimination rate constant: estimated slope of the linear regression of the natural log transformed concentrations vs time.
R^2_{adj}	Coefficient of determination of the line describing the terminal elimination phase, adjusted for the number of points
$t_{1/2}$	Terminal elimination half-life calculated as: ln 2/λz.
CL/F	(Apparent) total body clearance, calculated as: Actual dose/AUC.
Vz/F	(Apparent) volume of distribution calculated as: CL/F/λz.

Pharmacodynamics

The following PD parameters were determined for ristocetin cofactor (RICO) for each subject and each treatment period:

Time to Onset	Time point in hours of the first time at which the RICO measurement decreased below the threshold of 20%. Subjects for whom the RICO measurement did not decrease below 20% were censored at the last available assessment of RICO in the treatment period (pre-dose in Part 2 for Part 1 and FU for Part 2).
Time to Offset	Time point in hours at which the RICO measurement rose after onset and reached the threshold of 20%. Subjects who did not achieve Time to Onset or for whom the RICO measurement did not rise to 20% again were censored at the last available assessment of RICO in the treatment period (pre-dose in Part 2 for Part 1 and FU for Part 2).
Duration	Interval (h) between the times of onset and offset. Where a subject did not achieve onset, the Duration was 0 h. Where a subject achieved onset but not offset, the Duration was censored at the last available measurement as noted above.

The following PD parameters were determined for the von Willebrand factor antigen (vWF:Ag) for each subject and each treatment period:

AUEC	Area under the effect curve observed from time 0 h up to the last measurable data point computed using the linear trapezoidal rule.
AUEC_D	AUEC normalized to actual dose, computed as: AUEC/Actual Dose.
EMax	Value of the maximum effect directly obtained from the observed effect vs time curves; this maximum effect was the maximum decrease from baseline, for each treatment period.
EMax_D	EMax normalized to actual dose, calculated as: EMax/Actual Dose.
t_{max}	Time of the maximum effect directly

Safety

The evaluation of safety parameters comprised the analysis of: adverse events (AEs), laboratory variables (hematology, coagulation, clinical chemistry and urinalysis), vital signs, 12-lead electrocardiograms (ECGs), immunogenicity (anti-drug antibodies [ADA]) local tolerability findings and physical examination findings.

Statistical methods:

Statistical Analysis of Pharmacokinetic Data

The dose-normalized PK parameters C_{max}_D and $AUC_{0-\infty}$_D were analyzed using formal statistical analysis in a 2-stage mixed modeling approach. This bioequivalence analysis was to be performed with and without the flagged AUC0-∞_D values.

The mixed model included the actual treatment received, period and treatment sequence fitted as fixed effects, and subject nested within sequence fitted as a random effect.

In a first step, the presence of a carry-over was tested with this model by means of the treatment sequence, as in a 2 × 2 cross-over the treatment sequence and interaction of treatment and period are confounded.

If the treatment sequence was not statistically significant at the 10% significance level in any of the models, the carry-over was considered to be absent in each case.

Bioequivalence was then assessed using the first mixed model mentioned above.

Point estimates and 90% confidence intervals (CIs) for the comparison of the liquid product (i.e. Treatment B) and lyophilized product (i.e. Treatment A) were generated from the full model. These were back-transformed (i.e. exponentiated) to give model-adjusted geometric mean ratios (GMRs) and 90% CIs for the GMRs. The ratio was defined as Treatment A/Treatment B. P-values for the GMRs were presented as well as the adjusted geometric means for both formulations and intra-subject variability values for the comparison of the liquid and lyophilized products were presented. The 2 formulations were considered bioequivalent if the 90% CIs for the GMRs for both C_{max}_D and $AUC_{0-\infty}$_D for the comparison of the liquid and lyophilized product fell entirely within the acceptance interval (80%;125%).

The statistical analysis was performed using actual treatment received and actual sequence and was based on the PK population. The model was fitted using PROC MIXED in SAS, the method was specified as Restricted Maximum Likelihood and the denominator degrees of freedom for the fixed effects were calculated using Kenward and Roger's method.

Distributional assumptions underlying the statistical analyses were assessed by visual inspection of residual plots. Normality was examined by normal probability plots, while homogeneity of variance was assessed by plotting the residuals against the predicted values for the model.

Statistical Analysis of Pharmacodynamic Data

Analysis of AUEC_D for vWF:Ag and RICO Duration

Formal statistical analysis was performed on the AUEC_D for vWF:Ag and on the RICO Duration. These were analyzed following the same approach as that described for PK parameters.

Analysis of Time to Onset and Time to Offset

Each of the times of onset and offset for RICO was analyzed with a Cox proportional hazard regression model that included as factors the treatment, period and treatment sequence. In a first step the treatment sequence was tested. If the treatment sequence was not statistically significant in the models for time to onset or time to offset, the carry-over was considered to be absent. In a second step, hazard ratios, 90% CIs and p-values for the comparison of the liquid product (i.e. Treatment B) and lyophilized product (i.e. Treatment A) were generated from the full model. The hazard ratio was defined as Treatment A/Treatment B. The validity of the proportional hazards assumption was checked by using appropriate plots.

Analysis of Baseline Values

The baseline values of vWF:Ag were compared to evaluate the potential effect of Period 1.

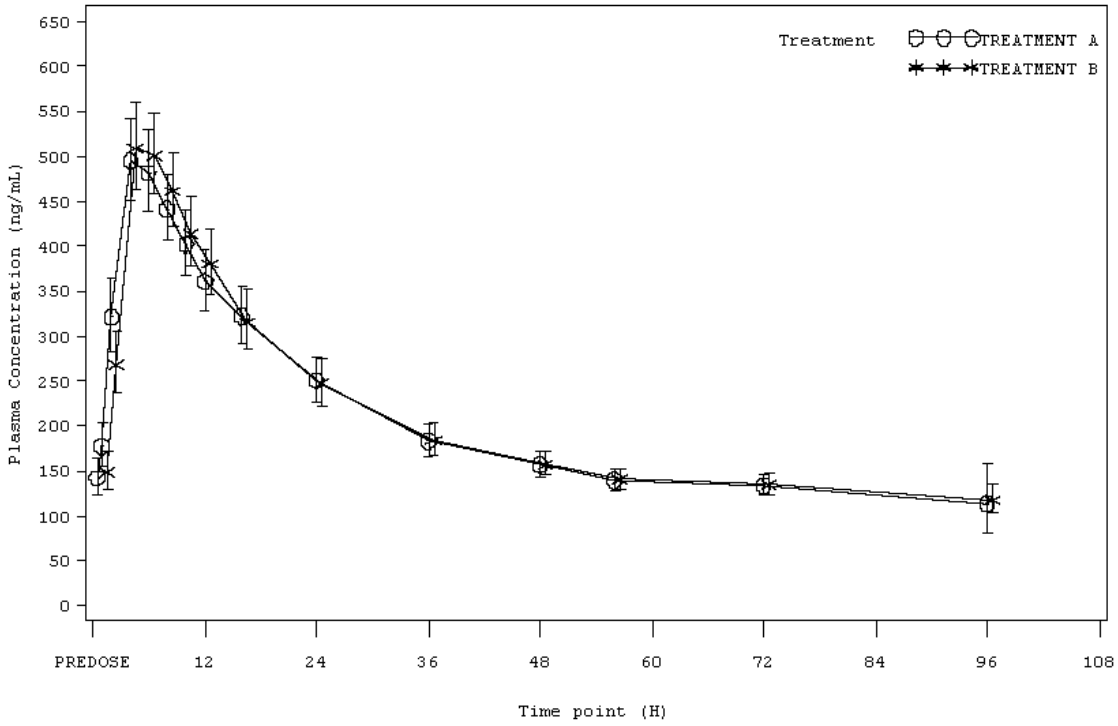
The mixed model that was used included treatment, sequence and period fitted as fixed effects and subject within sequence fitted as a random effect. A term for period had to be included in order for the 2 periods to be compared. The baseline values used in the analysis were untransformed and the mean difference in baseline values between Periods (Period 2 - Period 1) and its 90% CI were presented.

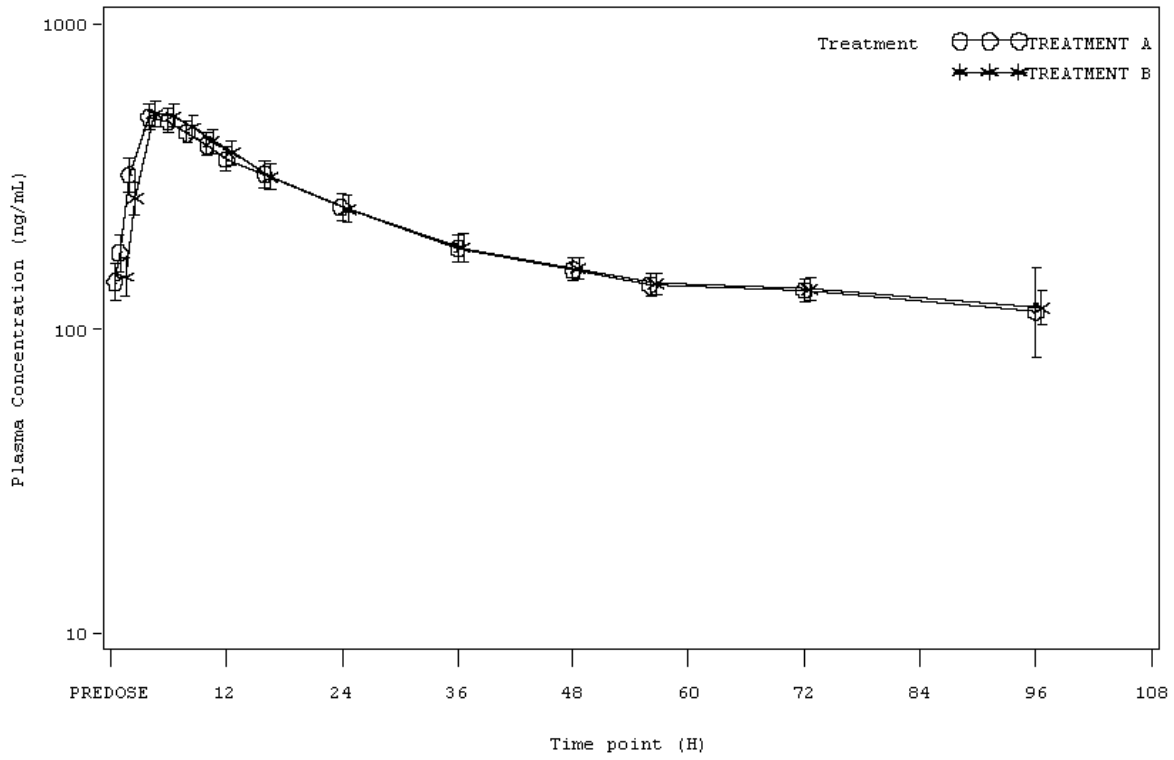
Summary Results:

Pharmacokinetic Results

The mean plasma concentration vs time profiles for Treatments A and B are illustrated on a linear and linear/log10 scale on the following page.

Mean (± SD) Plasma Caplacizumab Concentrations (ng/mL) by Treatment Presented on a Linear and Linear/Log$_{10}$ Scale





All values of LLOQ have been set to missing

Values are only presented up to 96 h time point as all values beyond 96 h are LLOQ

Treatment A (new formulation; reconstituted lyophilized drug product): single dose of 10 mg (0.8 mL) reconstituted lyophilized solution (12.5 mg/mL) of caplacizumab, administered by single SC injection

Treatment B (reference formulation; liquid formulation): single dose of 10 mg (2 × 1 mL) liquid formulation (5 mg/mL) of caplacizumab, administered as 2 SC injections of 1 mL each

Following SC administration of the 2 formulations of caplacizumab (Treatments A and B) the concentration vs time profiles were consistent with extravascular dosing. In addition, the concentration vs time profiles for caplacizumab tended to be similar in each subject for both treatments and the tmax values were similar following administration of both treatments. Overall, the geometric mean for C_{max}_D, $AUC_{0\text{-}last}$_D and $AUC_{0\text{-}\infty}$_D were similar for both treatments and this was confirmed by statistical analysis. The $AUC_{0\text{-}last}$_D values were also subdivided by the time of the last quantifiable concentration. The range of the occurrence of the last quantifiable concentration was similar for both treatments and resultant geometric mean values for each grouping were similar across treatments. It was also observed that the individual subjects had similar times for the last quantifiable concentration for each treatment. In addition, the $t_{1/2}$ was similar for both treatments.

Pre-dose data before the second treatment showed that the drug was completely washed out of all the subjects indicating that there was no carry-over effect.

The results for the bioequivalence analysis for the PK parameters $AUC_{0\text{-}\infty}$_D and C_{max}_D are presented below.

Dose Normalized Parameter	Treatment A		Treatment B					
	N	Adjusted Geometric Mean[a]	N	Adjusted Geometric Mean[a]	Ratio[b] (%)	90% CI[c] (%)	p-value[d]	CVw[e]

$AUC_{0-\infty}$_D (ng.h/mL/mg)	24	1967	24	1921	102.40	(97.77, 107.25)	0.39	9.4
C_{max}_D (ng/mL/mg)	24	48.1	24	49.4	97.22	(93.81, 100.75)	0.19	7.2

a Adjusted geometric means from mixed model
b Ratio of adjusted geometric means for Treatment A/Treatment B
c CI = confidence interval for ratio of adjusted geometric means
d p-value for ratio of adjusted geometric means
e CVw = intra-subject variability

Results obtained from mixed effect model including terms for actual treatment, sequence and period fitted as fixed effects and subject nested within sequence fitted as a random effect.

Results were considered bioequivalent if the 90% CI for the parameters $AUC_{0-\infty}$_D and C_{max}_D lie entirely within the range (80%, 125%).

Treatment A (new formulation; reconstituted lyophilized drug product): single dose of 10 mg (0.8 mL) reconstituted lyophilized solution (12.5 mg/mL) of caplacizumab, administered by single SC injection.

Treatment B (reference formulation; liquid formulation): single dose of 10 mg (2 × 1 mL) liquid formulation (5 mg/mL) of caplacizumab, administered as 2 SC injections of 1 mL each.

Pharmacodynamic Results

Following dosing with both formulations of caplacizumab, there was a rapid decrease in RICO levels. Levels fell below the threshold for complete RICO inhibition between 2 and 4 h after dosing, although the duration of inhibition varied between subjects, ranging from 22 to 70 h. All subjects had achieved Time to Offset by 72 h post-dose and mean plasma levels returned to the pre-dose level between 72 and 144 h post-dose. There was no notable difference in any of the parameters for vWF:Ag and coagulation factor VIII clotting activity (FVIII:C), or the Time to Offset for RICO between treatments. Time to Onset was statistically significantly earlier and Duration was statistically significantly longer following dosing with Treatment A compared to Treatment B. However, the observed differences were not considered to be clinically relevant. In addition, these results should be interpreted with caution as the sampling schedule which was optimized for the PK assessment might not be adequate enough to measure accurately Time of Onset.

The drop in RICO after dosing with caplacizumab indicated that the target pharmacological effect of a sustained inhibition of vWF mediated platelet aggregation for ≥24 h was reached for both Treatments A and B. This effect was accompanied by an expected decrease in vWF:Ag and coagulation factor VIII (FVIII) activity. There was a fast and reversible decrease in vWF:Ag and FVIII:C levels. For both PD biomarkers, pre-dose values were restored by 72 h post-dose and hence washout of the PD effect of caplacizumab could be considered effective. Furthermore, baseline levels of vWF:Ag in both treatments periods were equivalent.

For all 3 PD biomarkers, the results were as expected based on the pharmacology of caplacizumab and findings from previous clinical studies.

Safety Results

Single 10 mg doses of caplacizumab were well tolerated when administered by SC injection as a novel reconstituted lyophilized solution (Treatment A), and as the reference liquid formulation (Treatment B). There were no deaths or serious adverse events (SAEs) reported during the study and there were no CS findings in any laboratory assessments, vital signs or ECGs. Furthermore, no subject reported an abnormal CS physical examination finding that was considered related to investigational medicinal product (IMP) and no subject had a treatment-emergent ADA response.

A total of 20 AEs were reported by 11 (45.8%) subjects during the study, with fewer AEs reported by subjects receiving Treatment A compared to Treatment B (3 [12.5%] subjects compared to 10 [41.7%] subjects, respectively). The number of subjects reporting IMP-related AEs was relatively low. The most common IMP-related AE across both treatments was injection site erythema reported by 3 (12.5%) subjects (1 [4.2%] and 2 [8.3%] subjects after dosing with Treatments A and B, respectively).

As with the incidence of AEs, IMP-related AEs were less prevalent in subjects receiving Treatment A than Treatment B (2 [8.3%] subjects compared to 7 [29.2%] subjects, respectively). With the exception of injection site erythema, no IMP-related AE was reported by more than 1 subject following dosing with either treatment. Apart from 1 severe unrelated AE of ankle fracture reported after dosing with Treatment B and 1 moderate, Grade 2 related AE of injection site erythema reported after dosing with Treatment A, all AEs were assessed as mild in severity. In addition, all AEs had resolved by the end of the study, with the exception of the aforementioned AE of ankle fracture which was assessed as recovering/resolving.
Issue date: 12-Jan-2023