28 June 2018
EMA/490172/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Cablivi

International non-proprietary name: caplacizumab

Procedure No. EMEA/H/C/004426/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

List of abbreviations

Ab	Antibody
ADA	anti-drug antibodies
ADAMTS13	a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13
AE	adverse event
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ATC	Anatomical Therapeutic Chemical
AST	aspartate aminotransferase
aTTP	acquired thrombotic thrombocytopenic purpura
AUC_D	area under the curve dose corrected
AUCSS	area under the curve at steady state
BED	biologically effective dose
CI	confidence interval
C_{max}_D	maximum concentration dose corrected
CSR	Clinical Study Report
CT	computed tomography
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EMA	European Medicines Agency
FVIII	coagulation factor VIII
FVIII:C	FVIII clotting activity
GCP	Good Clinical Practice
GP	Glycoprotein
HCP	host cell proteins
HV	healthy volunteer
IgG	immunoglobulin G
INR	international normalized ratio
IPC	in-process control
i.v.	Intravenous
ITT	intent-to-treat
kDa	kiloDalton
KM	Kaplan-Meier
LDH	lactate dehydrogenase
MAA	Marketing Authorization Application
MCB	master cell bank
MD	multiple dose
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximally tolerated dose
MW	molecular weight
NOR	normal operating range
od	once daily
PAR	proven acceptable range
OLE	open-label extension
PCI	percutaneous coronary intervention
PD	Pharmacodynamics
PE	plasma exchange
PIP	Pediatric Investigation Plan
PK	Pharmacokinetics
PP	per protocol
pre-Ab	pre-existing antibodies
PT	prothrombin time
RICO	ristocetin-induced cofactor

RIPA	ristocetin-induced platelet aggregation
SAD	single ascending dose
SAE	serious adverse event
s.c.	Subcutaneous
SCR	Screening
SD	standard deviation
SmPC	Summary of Product Characteristics
SMQ	standardized MedDRA query
SOC	system organ class
TE	treatment-emergent
TE ADA	treatment-emergent anti-drug antibodies
TEAE	treatment-emergent adverse event
TFF	tangential flow filtration
TIMI	thrombolysis in myocardial infarction
TTP	thrombotic thrombocytopenic purpura
UK	United Kingdom
ULN	upper limit of normal
ULvWF	ultra-large (or unusually large) von Willebrand factor
US	United States
VH	heavy chain variable region
WCB	working cell bank
vWF	von Willebrand factor
vWF:Ag	von Willebrand factor antigen
WFI	water for injections

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Ablynx NV submitted on 3 February 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Cablivi, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 1 April 2016.

Cablivi was designated as an orphan medicinal product EU/3/09/629 on 30 April 2009 in the following condition: treatment of thrombotic thrombocytopenic purpura.

The applicant applied for the following indication:

'Cablivi is indicated for the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTTP).'

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Cablivi as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/Find medicine/Human medicines/European public assessment reports. (http://www.ema.europa.eu/ema/index.jsp?curl=/pages/medicines/human/medicines/004282/human_med_002276.jsp&mid=WC0b01ac058001d124)

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0189/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0189/2016 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance caplacizumab contained in the above medicinal product

to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol assistance

The applicant received Protocol assistance from the CHMP:

Scientific advice	Date	Area
EMEA/H/SA/1402/1/2009/PA/SME/III	6 January 2010	quality, non-clinical, clinical
EMEA/H/SA/1402/1/FU/1/2011/PA/SME/III	15 December 2011	quality, non-clinical, clinical

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Filip Josephson Co-Rapporteur: Joseph Emmerich

The application was received by the EMA on	3 February 2017
The procedure started on	23 February 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	16 May 2017
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	17 May 2017
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	29 May 2017
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 June 2017
The applicant submitted the responses to the CHMP consolidated List of Questions on	24 November 2017
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	4 January 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	11 January 2018
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	25 January 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	29 March 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	23 May 2018
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	N/A

The CHMP agreed on a 2nd list of outstanding issues to be sent to the applicant on	31 May 2018
The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on	5 June 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the 2nd List of Outstanding Issues to all CHMP members on	14 June 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Cablivi on	28 June 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Cablivi is indicated for the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTTP), in conjunction with plasma exchange and immunosuppression.

2.1.2. Epidemiology

Acquired TTP is a life-threatening, autoimmune blood clotting disorder manifested by microvascular occlusions and consequent thrombocytopenia, haemolytic anaemia, and organ ischemia. It is a rare disease with an incidence of 1.2 to 11 cases per million per year (Miller et al., 2004, Terrell et al., 2005, Reese et al., 2013, Kremer Hovinga et al., 2017). In the EU, the annual incidence of aTTP has been reported to be between 1.5 and 6 cases per million per year (Miller et al., 2004, Scully et al., 2008, Hassan et al., 2015, Veyradier et al., 2015). The incidence in children (<18 years) is much lower, about 3% of that in adults (Reese et al., 2013).

aTTP generally occurs in adulthood and is more common in women (2:1 vs men) and people of African descent. The median age at diagnosis is approximately 40 years.

2.1.3. Aetiology and pathogenesis

Acquired TTP is caused by inhibitory autoantibodies to ADAMTS13, resulting in a severe deficiency in this vWF-cleaving protease. Decreased ADAMTS13 activity leads to an accumulation of ULvWF multimers which bind to platelets and induce adhesion. The consumption of platelets into these microthrombi causes severe thrombocytopenia, tissue ischemia and organ dysfunction, commonly involving the brain, heart, and kidneys, and resulting in acute thromboembolic events such as stroke, myocardial infarction, and venous thrombosis, and in early death.

2.1.4. Clinical presentation, diagnosis and prognosis

With current treatment, episodes of aTTP have been reported to be associated with an acute mortality of up to 20%. Most deaths occur within 30 days of diagnosis and a median time to death of 9 days. Refractoriness to therapy (defined as a lack of improvement of the thrombocytopenia after 7 days of standard treatment, or as a failure of platelet count doubling after 4 days of standard therapy together

with elevated LDH levels) has been identified as an indicator of a poor prognosis for survival. The incidence of refractoriness is approximately 17% and is associated with a mortality rate reported to be as high as 42%.

In addition to the acute risks of the disease, patients experiencing an episode of aTTP may suffer long-term consequences such as cognitive deficits, depression, and arterial hypertension, and are at risk for recurrence. The reported recurrence rate ranges from 10-84%. Recurrences typically occur within 1-2 years but have been reported up to 30 years after the initial episode.

2.1.5. Management

A combination of plasma exchange (PE) and immunosuppressants (corticosteroids and increasingly also rituximab) is the mainstay of treatment. Currently no products have been authorised for the treatment of aTTP worldwide.

Plasma exchange, in which a patient's blood plasma is removed through apheresis and is replaced with donor plasma, removes ULvWF and the circulating autoantibodies against ADAMTS13 and replenishes blood levels of the enzyme. Plasma exchange should be started within 24 hours of presentation, as delay decreases the chance of response. Prior to the introduction of PE treatment, TTP was associated with extremely high mortality (~90%). Although PE has substantially improved survival, in spite of greater understanding of disease pathogenesis and the use of newer immunosuppressants, the mortality rate has not been further impacted.

Though considered a relatively safe procedure, in acutely ill patients such as those with aTTP, PE is often associated with major complications which may be related to the central venous catheter (e.g. infection, thrombosis, catheter insertion complications) or be plasma related (e.g. allergic reactions, alkalosis, volume depletion complications, infection). According to the Oklahoma TTP-HUS Registry, 24% of patients receiving PE followed between 1996-2011 experienced major PE related complications. Of note, a greater frequency of major PE related complications is observed among patients with ADAMTS13 activity of less than 10%, which may be related to a greater number of days that PE treatment is required. Therefore, treatment options that result in a reduction in the days and volume of PE would offer a clear advantage from a safety perspective.

Glucocorticoids are often administered as adjunct to PE in the initial treatment of aTTP and are maintained for a period of 1-2 weeks after its discontinuation. The use of corticosteroids is based on historical evidence that some patients with limited symptoms might respond to corticosteroids alone. There have been no studies specifically comparing the combination of PE with corticosteroids, versus PE alone.

Other immunosuppressive agents such as rituximab, a monoclonal anti-CD20 antibody that targets B-cell populations and reduces formation of inhibitory autoantibodies to ADAMTS13, are increasingly used as these are considered to address the underlying autoimmune process. Nevertheless, a limitation of rituximab treatment is its delayed onset of effect, with at least 3-7 days needed to achieve adequate B-cell depletion, and even longer to restore ADAMTS13 activity levels. In an acute disease setting, where immediate and effective intervention may be critical for survival and prevention of short term and long term damage, a rapidly acting medicine that offers immediate protection is needed.

Close monitoring of clinical symptoms and regular measurement of platelet counts guide the duration and intensity of PE and immunosuppression. Success of therapy is defined by platelet counts $\geq 150 \times 10^9$/L persisting over 48 hours. This is normally accompanied by at least partial recovery of ADAMTS13 activity.

Severe ADAMTS13 deficiency of <10% has been recognised as a biomarker in the acute and long-term management of patients with aTTP. During an acute episode, low ADAMTS13 activity levels may guide the intensity or duration of PE and the dose and type of immunosuppressants used. Patients experiencing severe ADAMTS13 deficiency of <10% during remission are at a 3-fold increased risk of recurrent disease compared to patients without such a deficiency. By using ADAMTS13 activity data to tailor therapy for these patients (e.g., pre-emptive use of rituximab), recurrence may be reduced.

The current standard of care treatment does not address the pathophysiological platelet adhesion leading to the formation of microthrombi. Other limitations are slow onset of efficacy and lack of efficacy in refractory patients. Thus, there remains a significant unmet medical need for rapid-onset therapies directly inhibiting the formation of microthrombi and tissue ischemia.

About the product

Caplacizumab is a humanised bivalent Nanobody that consists of two identical, genetically linked, humanised building blocks targeting the A1-domain of von Willebrand factor and inhibiting the interaction between von Willebrand factor and platelets. As such, caplacizumab prevents the ultralarge von Willebrand factor-mediated platelet adhesion, which is characteristic of aTTP. It also affects the disposition of von Willebrand factor, leading to transient reductions of total von Willebrand factor antigen levels and to concomitant reduction of factor VIII:C levels during treatment.

Based on its mode of action, caplacizumab presents an increased risk for mucocutaneous bleeding and potential prolonged bleeding after surgical interventions.

The first dose of Caplacizumab should be given as an intravenous injection of 10 mg prior to plasma exchange.

The subsequent doses should be given daily with a subcutaneous administration of 10 mg of caplacizumab after completion of each plasma exchange for the duration of daily plasma exchange treatment, followed by daily subcutaneous injection of 10 mg of caplacizumab for 30 days after stopping daily plasma exchange treatment.

If at the end of this period there is evidence of unresolved immunological disease, it is recommended to optimise the immunosuppression regimen and continue daily subcutaneous administration of 10 mg of caplacizumab until the signs of underlying immunological disease are resolved (e.g. sustained normalisation of ADAMTS13 activity level).

In the clinical development program, caplacizumab has been administered daily for up to 65 days. No data on re-treatment with caplacizumab are available.

2.2. Quality aspects

2.2.1. Introduction

Caplacizumab is a humanised bivalent Nanobody consisting of two identical building blocks joined by a tri-alanine linker. Nanobodies represent a novel therapeutic class of proteins derived from the heavy chain variable domains that occur naturally in heavy chain immunoglobulins from *Camelidae*. Caplacizumab is produced in *E. coli*.

Cablivi is presented as follows:

- Lyophilised powder: vial containing the active substance (strength 10 mg) formulated with sucrose, polysorbate 80, citric acid anhydrous and trisodium citrate dihydrate (pH 6.5);

- Solvent: pre-filled syringe containing water for injections.

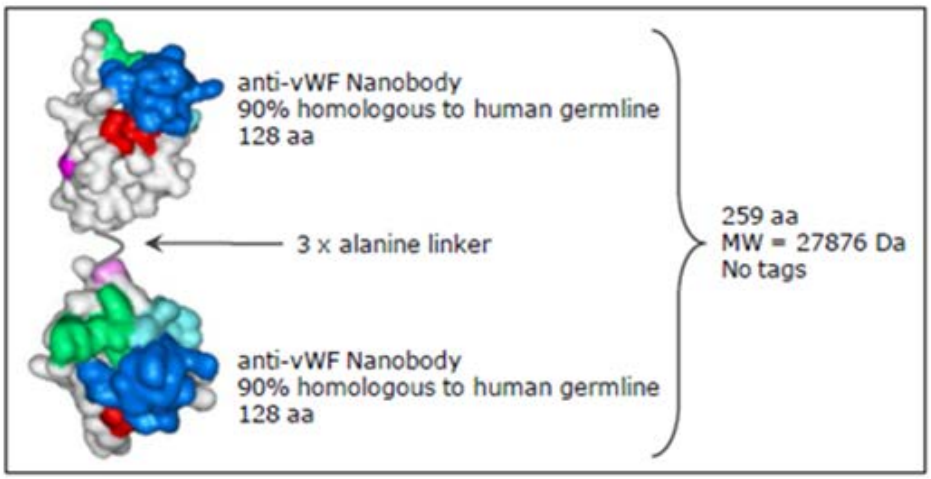
Cablivi is administered by intravenous and subcutaneous injection and is for single use only. Cablivi is supplied with 1 vial with the powder, 1 pre-filled syringe with the solvent, 1 vial adapter, 1 hypodermic needle and 2 alcohol swabs.

2.2.2. Active Substance

General Information

Caplacizumab consists of 259 amino acids and has a molecular weight of 27876 Dalton. A schematic representation is shown in Figure 1.

Figure 1*: Schematic structure of* caplacizumab *(ALX-0081)*

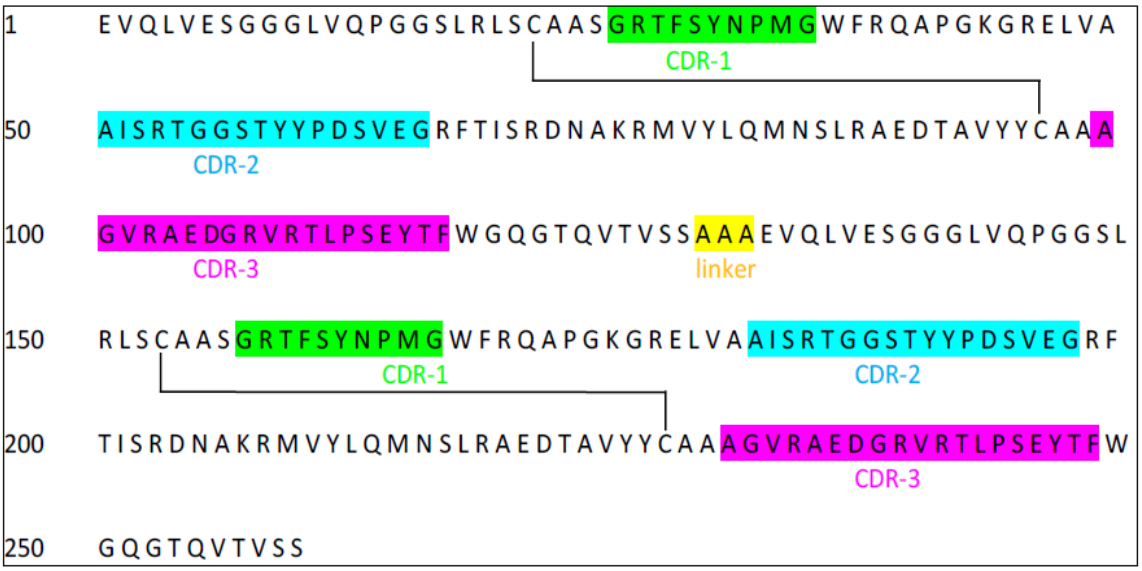


CDR-loops are colored as follow: CDR1 in green, CDR2 in cyan and CDR3 in blue. Red and purple residues refer to hallmark residues which are different for a VHH compared to a human VH. CDR: Complementarity Determining Region

The amino acid sequence of ALX-0081 is provided in Figure 2.

Figure 2: ***Amino acid sequence of ALX-0081***

The tri-alanine linker sequence connecting the two Nanobody building blocks is shown in yellow. The complementarity-determining regions (CDR-1, CDR-2 and CDR-3) of each subunit are shown in green, blue and magenta respectively. Disulfide bridges are shown as solid line.



Caplacizumab binds to the A1 domain of von Willebrand Factor (vWF), a key protein in haemostasis, and specifically interferes with the interaction of vWF with platelets. Caplacizumab is able to interact with vWF in both its active and inactive form, thereby preventing vWF-mediated platelet adhesion. The biological activity of caplacizumab is assessed using a Biacore potency assay to quantify binding of caplacizumab to vWF.

Manufacture, process controls and characterisation

Description of manufacturing process and process controls

Information about the facilities for manufacturing, storage and control of active substance has been provided in the dossier. GMP compliance has been demonstrated for all manufacturers.

The caplacizumab active substance is produced in Richter-Helm BioLogics (RHB), Germany (RHB), Dengelsberg, 24796 Bovenau, Germany. Caplacizumab active substance is expressed in *E. coli.* One vial of the working cell bank (WCB) is thawed and the cell culture is expanded in a seed fermentor. The expanded inoculum is transferred to a production fermentor. Expression of protein caplacizumab is induced. The harvest is clarified by tangential flow filtration (TFF) and dead-end filtration.

The purification process includes chromatographic capture, intermediate purification and polish steps followed by ultrafiltration (UF) and diafiltration (DF) steps. The purified material is then pooled and formulated followed by bioburden reduction filtration and filling. Caplacizumab active substance is shipped to the manufacturing site for finished product.

Caplacizumab active substance is stored in sterile bags. A certificate of analysis for the bags demonstrates the specification criteria (including cytotoxicity test, physiochemical test, biological reactivity test). A certificate of irradiation has also been provided. Compatibility of the container is evaluated in the stability section.

The Applicant is currently performing a dedicated extractable and leachable study on caplacizumab DS in its primary container closure system. The Applicant is recommended to provide the results of this study.

No reprocessing is claimed for the active substance manufacturing process.

The upstream and downstream processes are well described with large amounts of detail. Flowcharts for each step are provided, listing process in-depth descriptions, process parameters, set points and ranges. The critical process parameters (CPP) are indicated. The batch numbering system was provided.

Control of materials

Source, history and generation of the cell substrate

The origin of the cell substrate and detailed information on the development of the production cell line was provided. Development of the caplacizumab production strain involved the following steps:

• Immunisation

• Clone selection

• Formatting of humanised nanobodies into bivalent forms and lead selection

• Construction of production plasmid and generation of Research Cell Bank (RCB)

• Generation of Master Cell Bank (MCB)

• Generation of the Working Cell Bank (WCB)

The analytical methods used to characterise the different cell banks (MCB, WCB, end-of-production cell bank (EOPCB) and post-production cell bank (PPCB)), in line with ICH guidelines, are described in the dossier. The analysis chosen is suitable for the purpose.

The long-term stability program for the MCB and WCB was described.

A list of all raw materials used in the active substance manufacturing process (including filters and resins) is provided. Raw materials are of pharmacopoeial grade when possible. Non-compendial raw materials are controlled by internal specifications. All raw materials are purchased from qualified vendors. Written procedures are in place for receipt, identification, storage, handling, sampling, testing and approval or rejection of raw materials. These procedures provide assurance that materials are maintained in a controlled manner throughout the quarantine and release. Only raw materials and excipients certified as of non-animal origin are used for manufacture of caplacizumab active substance and finished product, except IPTG, which uses lactose as a starting material.

Composition of medium, solutions and buffers are also provided.

Filter materials and chromatography resins are also presented

Control of critical steps and intermediates

Lists of in-process controls (IPC), together with the corresponding acceptance criteria, for both the upstream and downstream processes, are provided. The control of critical steps and intermediates has been sufficiently described.

Process validation

Validation of the caplacizumab active substance process was performed using consecutive commercial-scale batches manufactured at RHB. Similar to the process description, the validation of the upstream process is described with a large amount of detail and data, being considered acceptable. There were some deviations in the upstream part of the validation runs. These are described and justified by the Applicant.

The downstream process validation was, similar to the upstream process validation, presented with a full data set of parameters and outputs. Profiles of chromatograms have been provided.

Clearance of process-related impurities, product-related impurities and product–related substances has been demonstrated, both graphically and in tabular form. The clearance of impurities is agreed upon.

Manufacturing process development

Three processes have been used during the development history of caplacizumab. Details about process differences, justification for making the changes, and results from comparability programs are provided. Batch release data, with corresponding acceptance criteria are provided in tabular form and compared for the different processes.

The major changes implemented in the final manufacturing process was the shift from MCB to WCB, removal of selective pressure, change in harvest technology and finally a change of formulation buffer and caplacizumab concentration to allow for production of a lyophilised finished product. The extended comparability program demonstrated some differences between processes; however, overall, comparability was demonstrated.

Qualification of small scale models have been presented in the dossier. The results are in general comparable, concluding that the small scale models are representative of the large scale manufacturing process. A few differences are described and justified. This is considered acceptable.

Characterisation of the upstream process was conducted using a design of experiment (DoE) set up. Although some of the investigated parameters were shown to influence caplacizumab product yield and quality, the evaluated ranges still resulted in acceptable caplacizumab quality.

Characterisation of the downstream process has been summarised in the dossier and further presented with a high degree of detail in four step reports.

Characterisation

Caplacizumab is a humanised bivalent nanobody consisting of two identical building blocks, each with one canonical disulfide bridge, joined by a tri-alanine linker. Caplacizumab binds to the A1 domain of von Willebrand Factor (vWF).The biological activity of caplacizumab is assessed using a Biacore potency assay to quantify binding of caplacizumab to vWF.

An extensive list of analytical methods has been applied for structural and biological characterisation of caplacizumab. The majority of the characterisation has been performed on the current master and working standards. However, additional engineering, clinical phase III and process performance qualification (PPQ) batches have also been used in several characterisation evaluations.

In general, the methods chosen and the results provided demonstrate that caplacizumab has been well characterised.

Three different assays were evaluated to investigate the biological activity. A fourth assay was used to determine the affinity of caplacizumab to human multimeric vWF. These methods are considered appropriate and reflect the mode of action.

A broad characterisation program has been performed for caplacizumab impurities, using several analytical methods in parallel during characterization of each impurity. Forced degradation studies have been executed and data both in tabular and graphical form has been provided. Furthermore, a potential loss of caplacizumab potency under stressed or accelerated conditions or during force degradation studies has been evaluated, in relation to each impurity.

The most pronounced product-related variants for caplacizumab are amino acid isomerisation, pyroglutamate, methionine oxidation and incorporation of norleucine. The product-related variants have been acceptably characterised.

Process-related impurities include host cell proteins (HCPs), DNA, endotoxins, polypropylene glycol (PPG), IPTG, glycine, antibiotics and elemental impurities. Process-related impurities controlled during release testing include residual HCPs, DNA and endotoxins.

Adequate clearance of process-related impurities has been validated during process validation. In addition, small-scale characterisation studies have demonstrated that both the intermediate and polish step have additional capacity to remove DNA and endotoxins.

Specification

The specification of the caplacizumab active substance includes control of identity, purity and impurities, potency and other general tests.

The proposed specification has been adequately justified and is considered acceptable. The major degradation products (product-related substances and impurities), noted during the stability studies, are tested within the active substance release specification.

The Applicant has justified the use of a single potency method claiming its overall superior analytic performance and its ability to identify stress-induced product-related variants compared to other methods.

The Applicant has justified the specification limits based on both active substance and finished product batches, from clinical and engineering batches. Finished product batches from the completed phase III study Hercules, in addition to several other (non-clinical) batches using the commercial manufacturing process are included. Results from non-clinical batches are considered as supportive. Nevertheless, the proposed specifications are all acceptable as the limits proposed are in line with the data from clinical phase III batches.

The proposed acceptance criteria for pH, osmolality, protein concentration, HCP, host cell DNA and endotoxins are acceptable.

Analytical methods

All analytical methods used for testing of the active substance are described in the dossier. At large, analytical methods are well described and their suitability proven using method validation according to ICH. In all validation studies, formulation buffers have been used to demonstrate that the formulations do not interfere with the results.

Potency and identity are determined by a method measuring the binding capacity to immobilised human von Willebrand Factor A1 domain (vWF-A1. The potency of a sample is expressed as percentage relative to an appropriate reference.

Batch analysis

Batch release analysis results from three GMP batches (used during PPQ, stability studies and clinical phase III studies) have been provided. Batch release data from several other historical batches manufactured with previous process versions are provided in the dossier. The results are comparable and acceptable.

Reference materials

A two tiered approach is used. The current master reference standard is lyophilised and used to assess stability of the working reference standard (WRS) and to bridge from one WRS to the next. The current liquid WRS is used in routine tests.

Future WRSs are tested according to a protocol containing predefined qualification criteria. Furthermore, a qualification plan for future master reference standards has been provided, including information about the potency calibration approach.

Stability

Stability data from three representative caplacizumab batches stored for ≥36 months are presented. All three batches remained within the current acceptance criteria up to 36 months. No significant changes or trends have been observed.

A shelf life of 36 months when stored at long-term conditions is acceptable for the active substance.In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Composition

The Cablivi finished product is supplied as powder and solvent for solution for injection:

- Powder: Type I glass vial with a stopper (butyl rubber), a seal (polypropylene) and a cap (polypropylene), containing 10 mg of caplacizumab.
- Solvent: Pre-filled syringe (type I glass cartridge closed with a bromobutyl rubber stopper) with 1 mL of water for injections used for reconstitution.

All container closure materials comply with Ph. Eur. requirements.

The pack size contains:

- 1 vial with powder

- 1 pre-filled syringe with solvent

- 1 vial adapter

- 1 hypodermic needle (30 gauge)

- 2 alcohol swabs

The composition of Cablivi consists of a 20 mM citrate buffer (pH 6.5), 7.0% sucrose (w/v) and 0.01% polysorbate 80 (v/v). The excipients are commonly used in the formulation of biopharmaceuticals.

The finished product is reconstituted with 1 mL WFI to allow for administration of 10 mg of caplacizumab. This takes into account a potential loss of active substance during reconstitution and

injection. This is found acceptable since an overfill is always necessary in order to be able to guarantee administration of the nominal dose.

For both intravenous and subcutaneous administration, the powder contained in the vial should be reconstituted using the vial adapter and all solvent in the pre-filled syringe. The solvent should be added slowly and mixed gently to avoid foaming of the solution. The vial with connected syringe should be allowed to stand on a surface for 5 minutes at room temperature.

The reconstituted solution is clear, colourless, or slightly yellowish. It must be visually inspected for particulate matter. A solution exhibiting particulates should not be used.

The entire volume of the reconstituted solution should be transferred back to the glass syringe and the entire volume of the syringe should be immediately administered.

Cablivi is for single use only. Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

The Applicant provided declarations of conformity for the devices.

Pharmaceutical development

The development of the lyophilised formulation is thoroughly described in the dossier. This formulation is used in the finished product process used for manufacture of clinical trial and future commercial supply.

The development of the manufacturing process is described in sufficient detail. The development of the control strategy is well explained and acceptably justified. The commercial finished product manufacturing process is a lyophilisation of 1 mL of active substance solution. It does not contain any additional compounding steps. The design and development of the lyophilisation process have been satisfactorily described with a sufficient degree of detail for all steps in the lyophilisation cycle.

The Applicant is currently performing a dedicated extractable and leachable study on Cablivi finished product in its primary container closure system. The Applicant is recommended to provide the results of this study when available.

Manufacture of the product and process controls

Manufacture of the lyophilised finished product

The Cablivi finished product manufacturing process consists of thawing and dispensing, pooling and mixing, pre-filtration and sterile filtration of caplacizumab bulk active substance, followed by filling, partial stoppering, lyophilisation, stoppering, crimping of glass vials, visual inspection, pack-off and storage. There are no declared hold points, but the product is held at various points for normal processing, and no reprocessing is permitted during the Cablivi finished product manufacturing process. Active substance batches can be pooled for the manufacture of one batch of the finished product.

The batch numbering system has been described.

Process validation

Process validation studies are based on a traditional approach and are performed using consecutive commercial-scale batches. All validation batches complied with the established in-process and release specifications, no critical deviations were observed.

The maximum processing time from start of pooling to start of lyophilisation has been covered in the process validation.

The aseptic filling has been sufficiently validated with media fills covering the maximum duration of filling.

The capability of the sterilising filter to reduce bioburden under worst case load conditions was shown by means of a bioburden challenge test by the filter vendor according to the vendor's protocol and corresponding report. The freeze-drying step has been sufficiently validated, in-process testing on samples from different positions at the shelves show a homogenous residual moisture level supporting the performance of both freeze dryers at the proposed batch size range. Shipping validation of final product from the finished product manufacturing site to the storage facility has also been acceptably performed.

Product specification

A) Cablivi finished product

The specification of the caplacizumab finished product includes control of identity, purity and impurities, potency and other general tests.

Analytical methods for Cablivi finished product

All analytical methods used for testing of the finished product are described in the dossier. Many test methods used for release testing and stability testing of the finished product are the same as those used for release testing and stability testing of the active substance. Additional method validation for finished product testing is not required given that the formulation of the reconstituted finished product is representative of that of active substance.

Batch analysis for Cablivi finished product

The batch analysis data presented for the PPQ batches complies with the limits in the proposed finished product specification except for protein content. For future batches, protein content (mg) will be included for release instead of protein concentration (mg/mL), which is supported by dose uniformity testing.

Justification of specifications for Cablivi finished product

For many tests the same acceptance criteria are proposed for finished product and active substance and these are justified in the same way in the dossier.

B) Water for injections

The specification and methods currently in place for testing WFI pre-filled syringes are presented in the dossier, and comprises testing for appearance, conductivity, acidity/alkalinity, chlorides, nitrates, sulphates, ammonium, calcium and magnesium, particulate matter, extractable volume, bacterial endotoxins and sterility. The testing program has been designed to fully comply with the current ICH Pharmacopoeias. For the tests following the same test principle and required by more than one Pharmacopoeia, a scientific assessment was made in order to determine according to which Pharmacopoeia the test should be carried out. The rationale for test selection was in all cases the more rigorous test parameter(s) or acceptance criteria. In cases where such a scientifically based alignment was not achievable (e.g. due to a different test principle), all tests are carried out as required by the Pharmacopoeias.

The specification has been justified.

Batch analysis data is provided for the validation batches.

Stability of the product

A shelf life of 48 months when stored at 2-8 ºC is proposed. It is also claimed that Cablivi may be stored at a temperature not above 25°C for a single period of up to 2 months, but not beyond the expiry date and Cablivi should not be returned to refrigerated storage after storage at room temperature.

An overview of the stability studies, conducted in line with ICH, is presented in the dossier, indicating currently available data. The study is on-going for up to 60 months. Stability data up to 60 months is available for one GMP-batch of finished product and for two non-GMP batches at long-term conditions. Stability data up to 24 months is also available for one additional GMP batch at the long-term storage condition.

At long term storage, all test parameters remained within the specification limits and no significant changes were observed for any of the parameters tested for up to 60 months storage at 2-8 ºC. During accelerated and stressed storage, the formation of a product-related substance and increase of the moisture content are the main changes observed.

An initial reconstitution study was performed for 24 h at 25°C supporting storage in a polypropylene-based syringe and expulsion through a 30 G needle. In addition, an in-use stability study was performed with the provided kit components, demonstrating that storage of reconstituted Cablivi in the syringes for 24 h at 25°C and 40°C did not influence the quality of the material. The chemical and physical in-use stability of 4 h at 25°C, stated in the SmPC, is found acceptably justified.

Furthermore, in-use stability data support that the reconstituted product can be administered via a venous catheter in combination with commonly used perfusion lines and flush solutions.

Photostability studies were conducted and the results presented. Cablivi should be stored in the original package in order to protect from light.

The data presented support the proposed shelf-life of 48 months at 2-8°C as well as storage of the non-reconstituted product at a temperature not above 25°C for a single period of up to 2 months.

In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Comparability exercise for the finished product

The formulation of both active substance and finished product was changed from a phosphate to a citrate based buffer, combined with the change of the finished product presentation from a solution to a lyophilised powder. Additionally, the concentration of active substance and reconstituted finished product is higher compared to previous process versions.

It is claimed that the results of the analytical comparability assessment overall support the comparability and/or improvement of the commercial process as compared to previous process. However, similar to the active substance some differences were observed between processes. Nevertheless, despite this minor difference comparability has been acceptably demonstrated.

Adventitious agents

Non-viral adventitious agents

Microbial adventitious agents are controlled throughout the manufacture of caplacizumab active substance and finished product.

In accordance with the current version of the CHMP guideline "Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA/410/01), raw materials, product contact materials and excipients which present no BSE/TSE risk are used for the manufacturing of Only raw materials and excipients certified as of non-animal origin are used for manufacture of caplacizumab active substance and finished product, except IPTG, which uses lactose as a starting material. Lactose is isolated from bovine milk, subsequently transformed into galactose, and further into IPTG by a multistep chemical synthesis process, i.e. it is not derived from milk directly. According to EMEA/410/01 Rev. 2, milk and milk-derived products including lactose and galactose are unlikely to present any risk of BSE/TSE transmission.

A declaration of origin and TSE statement of the IPTG supplier was provided.

In addition, TSE statements of the caplacizumab drug substance manufacturer are provided in the dossier.

Viral adventitious agents

The host cell caplacizumab MCB and WCB used for the manufacture of caplacizumab active substance is an *E. coli* derivative. Based on the microbial nature of the manufacturing process and the materials used therein, the risk for viral contamination of caplacizumab can be excluded and the product is considered not to fall within the scope of ICH Q5A guideline on "*Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*".

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The Applicant provided a well-structured quality dossier resulting in a limited numbers of issues being raised and no Major Objection. All the issues have been resolved.

Active substance

The cell bank system is properly tested and qualified. The active substance manufacturing process is described in sufficient details. CPPs are identified and the active substance manufacturing process is appropriately validated.

Extended characterisation of caplacizumab has been performed. Multiple orthogonal analytical methods have been applied to assess caplacizumab molecular properties, such as primary structure, post-translational modifications, higher order structure, and biological activity.

The approach for setting active substance and finished product specification limits for potency, purity and product-related impurities was initially not agreed upon. The specifications were set based on a central value from a single finished product batch from a previous process version. The revised calculations for justification of specification limits for potency, purity, product related substances and product-related impurities, based on previous and current process batches are found acceptable.

Data submitted supports a shelf life of 36 months when stored at long-term conditions.

Finished product

The pharmaceutical development is found comprehensive and acceptably addressed. The control strategy, manufacturing process and the controls of critical steps are sufficiently described.

Data presented support the proposed shelf-life of 48 months at 2-8°C as well as storage at a temperature not above 25°C for a single period of up to 2 months.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Overall, the quality of Cablivi is considered to be in line with the quality of other approved monoclonal antibodies. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The cell culture and purification of the active substance are adequately described, controlled and validated. The active substance is well characterised with regard to its physicochemical and biological characteristics, using state-of-the-art methods, and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications.

Viral safety and the safety concerning other adventitious agents including TSE have been sufficiently assured.

The overall quality of Cablivi is considered acceptable when used in accordance with the conditions defined in the SmPC.

2.2.6. Recommendation for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended a point for investigation.

2.3. Non-clinical aspects

2.3.1. Pharmacology

Primary pharmacodynamic studies

The non-clinical pharmacological activity of caplacizumab was assessed using *in vitro* and *ex vivo* binding and potency assays as well as in a diseased-like model in baboons *in vivo*.

In vitro

Caplacizumab is a bivalent humanised Nanobody directed against the A1 domain of vWF. Binding of caplacizumab to the A1 domain of vWF inhibits interaction between vWF and the platelet GPIb-IX-V receptor. This neutralisation of vWF activity can be observed as a pharmacodynamic (PD) response by measuring a decrease in ristocetin cofactor activity (RICO PD assay). Target mediated disposition is observed during caplacizumab treatment as a reduction in plasma levels of vWF and FVIII (which has vWF as carrier molecule). The nonclinical development of caplacizumab showed that the Nanobody avidly binds to the A1 domain of human vWF *in vitro* (K_D = 3.76 pM) and inhibits potently ristocetin-induced binding of vWF to platelets (IC_{50} = 0.26 nM) in a PD assay. Results from *in vitro* perfusion chamber experiments demonstrated that caplacizumab selectively inhibits platelet adhesion at high shear rates, such as those observed in normal arterioles and stenotic arteries. Under these conditions, platelet adhesion was completely inhibited by ~ 0.4 µg/mL of caplacizumab. The antithrombotic effect of caplacizumab was further demonstrated in ex vivo perfusion studies, using blood from healthy individuals and PCI patients. For all PCI patients, complete inhibition of platelet adhesion *ex vivo* was observed when 0.8 µg/mL caplacizumab was spiked to the human blood. Results from a flow chamber experiment demonstrated that Caplacizumab blocked adhesion of platelets to ULvWF secreted from

endothelial cells in the presence of plasma from TTP patients. The effective caplacizumab concentration ranged from 0.2 µg/mL to 0.8 µg/mL and was correlated to the reduction of vWF levels in the patients' plasma.

Caplacizumab was shown to be cross-reactive with tissues expressing vWF (i.e. megakaryocytes and endothelial cells) from guinea pigs and monkeys, but the Nanobody was not cross-reactive for mouse, rat, dog and rabbit vWF.

Caplacizumab displays a similar *in vitro* pharmacology profile on human, cynomolgus monkey and guinea pig vWF. The monovalent building block of caplacizumab interacts with similar affinity to cynomolgus monkey vWF A1-domain (1 nM) compared to human vWF A1-domain (1 nM) and with 10-fold less affinity to guinea pig vWF A1-domain (10 nM). Complete in vitro target neutralization by caplacizumab, measured as vWF mediated platelet aggregation in the presence of ristocetin (RIPA assay), was obtained at 0.4 µg/mL and 1-2 µg/mL for human and cynomolgus monkey/ guinea pg vWF, respectively. Furthermore, a similar caplacizumab concentration was required in binding studies for full target occupancy (human: 1-3 µg/mL, cynomolgus monkey/ guinea pig 2-5 µg/mL). Cynomolgus monkey and guinea pig were thus selected as the relevant species for the pivotal toxicity studies.

In vivo

In an arterial thrombosis model (Folts model) *in vivo*, the caplacizumab plasma concentration for complete antithrombotic efficacy (inhibition of cyclic flow reduction), was between 0.3 and 0.5 µg/mL. Biomarker analysis using *ex vivo* RIPA analyses demonstrated that complete inhibition of ristocetin-induced PD response (platelet aggregation) correlated with the observation of complete antithrombotic efficacy.

The *in vivo* efficacy of caplacizumab was evaluated in a baboon model of TTP after preventive and therapeutic treatment. In this model mimicking the early onset of acquired TTP, the disease is induced by functional inhibition of ADAMTS13 through administration of an inhibitory monoclonal antibody (3H9). Caplacizumab (2.5 mg/kg/day, s.c., q.d. for 5-7 days; effective plasma concentration range: 145 - 407 ng/mL) was efficacious in preventing and treating typical features of an acute episode of aTTP (i.e. thrombocytopenia (drop in platelet counts) and schistocytic haemolytic anaemia (red blood cell fragmentation). The efficacy of caplacizumab in the aTTP model correlated with suppression of the RICO PD marker. This acute disease model has limitations as no information is available regarding the long-term effect and /or relapses of the disease. However, the lack of such preclinical information is considered superseded by clinical efficacy data in patients.

A sequential PK/PD modelling approach allowed the characterisation of the pharmacokinetics of caplacizumab in baboons, and of the relationship between exposure, vWF:Ag and platelet counts supporting the use of vWF:Ag as marker for efficacy. In terms of effects on bleeding and coagulation, the expected decrease in the vWF:Ag and FVIII:C levels in baboons after repeated administration of caplacizumab appears to have no obvious effects on bleeding and coagulation, as there were no effects on coagulation markers (PT, aPTT, TT and fibrinogen) and no signs of bleeding was revealed following brain CT scan or post-mortem analysis of different tissues.

Secondary pharmacodynamic studies

The specificity of caplacizumab was assessed in tissue cross-reactivity studies in various species. Immunohistochemical staining of human, *Cynomolgus* monkey and guinea pig tissues demonstrated that caplacizumab only binds to vWF expressing cells (i.e. megakaryocytes and endothelial cells) and caplacizumab did not bind to human blood cells or thrombocytes.

Further secondary pharmacology studies showed the absence of interference with vWF binding to collagen type VI, ADAMTS13 and fibrillary collagens. Moreover, caplacizumab did not interfere with the enzymatic activity of ADAMTS13 and caplacizumab did not affect the binding of FVIII to vWF. Caplacizumab showed no tissue cross-reactivity to mouse, rat, dog and rabbit. A general secondary pharmacology profiling panel for off-target activity was not provided considering that this is a biological product.

A potential bleeding risk associated with caplacizumab treatment was assessed in various models as well as by an analysis of coagulation tests in toxicity studies. At therapeutic doses, caplacizumab was shown to increase template bleeding times in baboons which were correlated with an increased blood loss from surgical wounds but with no obvious signs of spontaneous or internal bleeding. At higher doses administered in the repeat dose toxicity studies, occasional macroscopic bleeding was observed (see toxicology section).

Safety pharmacology programme

Safety pharmacology (clinical signs including respiratory and circulatory systems, somatomotor activity and behaviour patterns; body temperature; ECGs and blood pressure) was evaluated in the repeat-dose toxicity studies in *Cynomolgus* monkeys and guinea pigs after intravenous (up to 12 mg/kg/day) and subcutaneous (up to 40 mg/kg/day) administration. Safety endpoints were included (clinical signs' and visual observations) in the repeat-dose toxicity studies in order to address respiratory function and general behaviour in line with ICH S6 guideline for biotech derived products.

No *in vitro* safety studies such as hERG channel testing were submitted considering that the drug product is an antibody/Nanobody.

Overall, the safety pharmacology studies *in vivo* indicate that, besides bleeding, there are no adverse cardiovascular effects (ECG, blood pressure and heart rate) or any other findings concerning the safety parameters evaluated up to 40/mg/kg/day, yielding exposure margins of >20-fold to the predicted clinical exposure for the recommended dose of caplacizumab (10 mg/day, AUC=300 µg h/mL) as shown below:

Pharmacodynamic drug interactions

No dedicated pharmacodynamic drug interaction studies were conducted with caplacizumab considering that this is an antibody/Nanobody that specifically interacts with Von Willebrand factor.

2.3.2. Pharmacokinetics

The non-clinical pharmacokinetic properties of caplacizumab were analysed in guinea pigs and baboons as cross-reactive species, as well as in mice (not cross-reactive). No dedicated pharmacokinetic studies were performed in *Cynomolgus* monkeys, but assessment of single dose pharmacokinetics of caplacizumab was included in a single-dose toxicity study. Caplacizumab PK/TK after repeated administration and the formation of anti-drug antibodies (ADAs) was mainly investigated in toxicity studies conducted in guinea pig and *Cynomolgus* monkey. The distribution of caplacizumab radioactively labelled with ^{125}I was examined in mice.

Methods

The concentration of caplacizumab in guinea pig and *Cynomolgus* monkey plasm was determined by an ELISA-based method. In short, caplacizumab in the samples was captured in microtiter plates using a bivalent anti-caplacizumab Nanobody followed by incubation with vWF and detected via its binding to

vWF using purified polyclonal rabbit anti-vWF IgG. This ELISA method detects caplacizumab via its binding with vWF and as such, measures total active caplacizumab.

In all toxicology studies with exception of the 26-week *Cynomolgus* monkey study, ADAs were determined by an ELISA-based bridging format in which anti-caplacizumab antibodies were captured with caplacizumab adsorbed to the wells of a 96-well microplate. Bound antibodies were detected using biotinylated caplacizumab and horseradish peroxidase-labelled extravidin which allowed visualisation via a substrate tetramethylbenzidine (TMB).

For the 26-week toxicity study, an ADA assay with improved drug tolerance was developed as an in solution-phase kinetics based homogenous bridging assay (Gyrolab-based). Test samples were acidified to induce drug-antibody dissociation and improve tolerance towards free caplacizumab present in the samples. Subsequently, samples were neutralised in a mastermix containing an excess of biotinylated and Alexa Fluor 647-labeled caplacizumab. After binding of ADA to the labelled drug, the mix was transferred to neutravidin coated microstructures on compact disks CD's in the Gyrolab system. The amount of fluorescence per microstructure was detected with the laser-induced fluorescence detector incorporated in the Gyrolab platform.

Absorption

Caplacizumab shows a non-linear kinetic profile. In all species, a first phase characterised by a rapid decline in plasma levels can be described at the higher doses immediately after administration (i.v.) or after peak plasma levels are reached (s.c.). This is likely caused by rapid distribution of the free caplacizumab, in combination with elimination via renal clearance.

After i.v. administration in guinea pigs and *Cynomolgus* monkeys, Cmax increased in a dose linear manner, whereas the AUC decreased relative to the dose and a non-linear, less than proportional dose dependency was observed. The mean apparent terminal half-lives (t1/2) after i.v. administration ranged between 9 and 16 hours in guinea pigs, and between 8 and 37 hours in monkeys.

After s.c. administration, drug plasma profiles were characterised by absorption-controlled kinetics. Both Cmax and AUC increased with increasing doses, but less than proportionally in both guinea pigs and monkeys. Absorption following s.c administration was rapid in guinea pigs (t_{max} ranged between 2 and 4 hours), while slower in monkeys with t_{max} values ranging from 6 to 16 hours. The mean apparent terminal half-lives after s.c. administration was approximately 6 hours in guinea pigs and ranged between 16 and 27 hours in monkeys. The apparent bioavailability of caplacizumab after s.c administration was high, ranging from 79% to over 200% in guinea pigs and from 82% to 97% in monkeys.

Distribution

The *in vivo* biodistribution of ^{125}I-radiolabeled caplacizumab was assessed in mice. As mouse vWF does not interact with caplacizumab, two groups of animals were assessed - one treated with free ^{125}I-caplacizumab and the other treated with ^{125}I-caplacizumab in complex with human vWF to allow comparison of the two caplacizumab forms (free and vWF-complexed).

Upon injection of free ^{125}I-caplacizumab, the majority of the radioactivity was found in blood, kidneys and liver with little amounts of radioactivity observed in other organs. Radioactivity was observed in the stomach as similarly described for other proteins and attributed to accumulation of iodine. When a complex of ^{125}I-caplacizumab and human vWF was injected into the mice, the majority of radioactivity was found in the liver and in blood with minor amounts in the kidney. The results suggest that vWF-bound caplacizumab targets the liver. In contrast, unbound caplacizumab appeared to distribute to the kidneys. In pregnant guinea pigs, exposure to traces of caplacizumab in foetuses was demonstrated

indicating placental transfer of caplacizumab in this species. Foetal exposure to caplacizumab in primates and humans remains uncertain, as proteins lacking an Fc portion are not thought to freely pass the placental barrier.

Metabolism

No dedicated metabolism studies were performed.

Excretion

No dedicated excretion studies were performed, but data from the biodistribution study in mice using ^{125}I-radiolabeled caplacizumab suggest that free caplacizumab is eliminated via the kidneys, while vWF-complexed caplacizumab appears to target and possibly be catabolized in the liver. Therefore, in cross-reactive species, it is assumed that caplacizumab plasma concentrations exceeding the vWF levels are eliminated in the kidneys , while the caplacizumab-vWF complex displays a slower elimination and presumably follow the natural fate of vWF via hepatic catabolism. This assumption is supported by the dose-dependent plasma concentration versus time curve observed in cross-reactive species and the detection of caplacizumab in the urine of treated *Cynomolgus* monkeys.

2.3.3. Toxicology

The toxicity of caplacizumab was examined in compliance with ICH S6(R1). The toxicology of caplacizumab was evaluated in single and repeat-dose toxicity studies in both rodent (guinea pig) and non-rodent species (*Cynomolgus* monkey). All single and repeat dose toxicity studies were conducted in compliance with Good Laboratory Practice (GLP). Toxicokinetic investigations were performed in the repeat-dose toxicity studies in guinea pig and *Cynomolgus* monkey after both intravenous (i.v.) and subcutaneous (s.c.) administration. These animal species were considered appropriate for the non-clinical safety evaluation of caplacizumab based on characterization of affinity towards species vWF A1 domain, *in vitro* binding, functional assays and demonstrated *in vivo* pharmacodynamic effects. In the pivotal toxicity studies toxicokinetics and concurrent pharmaco-dynamic (PD) responses measured as neutralisation of von Willebrand factor (vWF) by RICO-analysis showed that the doses used were sufficient to reveal potential toxicological effects by caplacizumab. Full neutralisation of vWF activity, defined as a RICO activity lower than 20%, was demonstrated in *Cynomolgus* monkey throughout the treatment period for all treatment groups. For guinea pig, full pharmacodynamic effect on target neutralisation was shown for the intermediate and high dose group and partial inhibition was noted for the low dose group. This shows that caplacizumab is pharmacologically active in both species used for toxicity testing and that the animals were sufficiently exposed - for monkeys in all dose levels applied in repeat-dose toxicity studies - to provide complete target inhibition. Therefore, the species and the dose levels examined in toxicity studies were relevant as models for human safety assessment.

Table 1: Toxicity studies performed with caplacizumab.

Type of Study	Species/Strain	Route of Administration	Study Number	GLP[1]
Single-dose toxicity	guinea pig, Dunkin Hartley	intravenous bolus	LPT 20093	Yes
Dose-escalation study	Cynomolgus monkey	intravenous bolus	LPT 20094/06	Yes
Single-dose toxicity	Cynomolgus monkey	intravenous bolus or subcutaneous injection	LPT 22630	Yes
13-week repeat-dose toxicity	guinea pig, Dunkin Hartley	subcutaneous injection	LPT 24023	Yes
2-week repeat-dose toxicity	Cynomolgus monkey	intravenous bolus	LPT 20095	Yes
2-week repeat-dose toxicity	Cynomolgus monkey	intravenous bolus or subcutaneous injection	LPT 22631	Yes
13-week repeat-dose toxicity	Cynomolgus monkey	subcutaneous injection	LPT 24024	Yes
26-week repeat-dose toxicity	Cynomolgus monkey	subcutaneous injection	AA93337	Yes
DRF and TK in pregnant guinea pigs	guinea pig, Dunkin Hartley	intramuscular injection	LPT 25265	Yes
Embryo-fetal development (Segment II)	guinea pig, Dunkin Hartley	intramuscular injection	LPT 20179/06	Yes
Local tolerance after single administration	rabbit, Himalayan	intravenous, intramuscular, intra-arterial, paravenous and subcutaneous injection	LPT 20340/06	Yes

[1] All GLP-studies were conducted in OECD member states

Single dose toxicity

Single-dose toxicity studies were conducted with caplacizumab in guinea pigs and *Cynomolgus* monkeys by i.v. or s.c. routes.

Table 2: Single-dose toxicity studies

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dose Levels	Gender and No. per Group	Observed Maximum Non-Lethal Dose	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Guinea pig, Dunkin Hartley	intravenous (i.v.) bolus (D-PBS, 0.2 M Glycine, 0.02 % Tween 80; pH 7.1 ± 0.1)	0; 2 and 20 mg/kg	5M/5F per group	20 mg/kg	not observed	No signs of local or systemic toxicity. vWF or FVIII concentrations not measured. ADA testing not performed.	LPT 20093
Cynomolgus monkey	i.v. bolus (D-PBS, 0.2 M Glycine, 0.02 % Tween 80; pH 7.1 ± 0.1)	0; 7.5; 74.7 and 747 µg/kg	2M/2F	747 µg/kg	not observed	No signs of local or systemic toxicity. No ADA detected.	LPT 20094/06
Cynomolgus monkey	i.v. and subcutaneous (D-PBS, 0.2M Glycine, 0.02% Tween 80, pH 7.1 ± 0.1)	0; 0.02; 0.4 and 8 mg/kg	3M/3F per group	8 mg/kg	not observed	No signs of local or systemic toxicity. Statistically significant decrease of FVIII in M+F at 8 mg/kg s.c., in M at 0.4 mg/kg s.c. and in F at 8 mg/kg i.v. Statistically significant decrease of vWF in F at 8 mg/kg i.v. Treatment emergent ADA detected in the highest dose groups (2/6 s.c.; 1/6 animal i.v.).	LPT 22630

ADA: anti-drug antibodies; F: female; FVIII: coagulation factor VIII; M: male; vWF: von Willebrand factor

The single-dose toxicity studies conducted showed a low toxicity of caplacizumab following parenteral administration. Caplacizumab was evaluated following i.v. administrations up to 20 mg/kg in guinea pig and i.v. or s.c. administrations up to 8 mg/kg in *Cynomolgus* monkey with 2-week observation periods. No mortality or clinical signs of toxicity were observed in any of the animals in these studies. In addition, no caplacizumab related effects were observed for any of the animals regarding the body weight and body weight gain, the food and drinking water consumption and haematological parameters. Post-mortem macroscopic and histologic examination did not reveal any caplacizumab related changes.

Repeat dose toxicity

Repeat-dose toxicity studies were conducted with caplacizumab in guinea-pigs and *Cynomolgus* monkeys by i.v. or s.c. routes and designed to achieve continuous maximal target occupancy (target saturation) and maximal exposure using multiple dosing schemes. The selection of the highest dose used was guided by recommended dose volumes. Comparative mean systemic exposures reached in nonclinical studies and simulated clinical trials with caplacizumab are presented in the *Table 3*.

Table 3: Repeat-dose toxicity studies.

Type of Study	Species and Strain	Method of Administration	Dose Levels	GLP	Testing Facility	Study Number
13-week repeat-dose toxicity in guinea pigs	guinea pig, Dunkin Hartley	subcutaneous injection	**4 x** 10 mg/kg/day	Yes	LPT	LPT 24023
2-week repeat-dose toxicity in Cynomolgus monkeys	Cynomolgus monkey	intravenous bolus	**6 x** 0.006/0.0015; 0.6/0.2 and 6/2 mg/kg/day[5]	Yes	LPT	LPT 20095
2-week repeat-dose toxicity in Cynomolgus monkeys	Cynomolgus monkey	intravenous bolus or subcutaneous injection	i.v.: **6 x** 0, 2 mg/kg/day s.c.: **6 x** 0, 0.02, 0.4, 2 mg/kg/day	Yes	LPT	LPT 22631
13-week repeat-dose toxicity in Cynomolgus monkeys	Cynomolgus monkey	subcutaneous injection	**4 x** 10 mg/kg/day	Yes	LPT	LPT 24024
26-week repeat-dose toxicity in Cynomolgus monkeys	Cynomolgus monkey	subcutaneous injection	**3 x** 0; 0.135; 1.35 and 13.5 mg/kg/day	Yes	WIL Research, France	AA93337

Table 4: Comparative mean systemic exposures reached in nonclinical studies and simulated clinical trials with caplacizumab

A NOAEL was defined for the 26-week toxicity study in cynomolgus monkey, obtained safety ratios to predicted exposures in TTP patients are highlighted in bold. Exposure margins calculated for the remaining toxicity studies are listed for information.

STUDY	SPECIES	Daily dose (mg/kg)	$AUC_{0\text{-}24h}$ [a] (µg.h/mL) (AUC dosing interval)	RATIO AUC [b]
2-week toxicity study after s.c. administration (LPT 22631)	Cynomolgus monkey	12[e]	156 (26)	12.5
13-week toxicity study after s.c. administration (LPT 24023)	Guinea pig	40[d]	628 (157)	50.3
13-week toxicity study after s.c. administration (LPT 24024)	Cynomolgus monkey	40[d]	524 (131)	42.0
26-week toxicity study after s.c. administration (AA93337)	Cynomolgus monkey	**4[c]**	**300**	**24.1**
Simulated caplacizumab exposure (PK/PD modelling)	Human (aTTP patients)	10	12.473	–

a AUCSS,24h: calculated as the area under the curve at steady-state for 24h.

b Calculated AUC ratio with human as reference (20 mg daily dose).

c NOAEL was at 4 mg/kg daily dose in this study

d A NOAEL was not defined, exposures are based on the highest dose tested

e Exposure is predicted at the steady state for the typical TTP patient (70 kg body weight) for the highest dose administered in TITAN (20 mg daily).

Safety pharmacology endpoints were included in all repeat-dose toxicity studies, as well as local tolerance assessments. Assessment of male and female fertility parameters including functional measurements was included in the 13-week study in *Cynomolgus* monkeys.

Caplacizumab was generally well tolerated. No caplacizumab-related hepatoxicity or nephrotoxicity was observed in any of the repeat-dose toxicity studies. An increased bleeding tendency with concurrent decreases in plasma vWF:Ag and FVIII:C levels as well as changes in serum chemistry parameters related to the anti-thrombotic pharmacology of caplacizumab was observed in guinea pigs (haemorrhagic subcutaneous tissue at the injection sites) and *Cynomolgus* monkeys (haemorrhagic subcutaneous tissue at the injection sites, nose bleed, menorrhagia, hematomas at sites of animal handling or experimental procedures, prolonged bleeding at injection sites) at doses ranging from 0.04 to 12 mg/kg/day by intravenous and 0.4 to 40 mg/kg/day by subcutaneous administrations . These effects elicited by caplacizumab in guinea pig and *Cynomolgus* monkey were generally dose-dependent and reversible. Urinalysis showed increased urinary bilirubin levels at some occasions in the mid and high dose groups in the 13-week study in *Cynomolgus* monkey. This finding was not observed in the 26-week study with *Cynomolgus* monkey. The toxicokinetics data in both guinea pig and *Cynomolgus* monkey show that caplacizumab exposure varies between gender after both i.v. and s.c. administration. In the pivotal 26-week repeat-dose toxicity study in *Cynomolgus* monkey C_{max}- and AUC-levels were 1.5-1.7 times higher in males. NOAELs were set at the highest doses tested in all studies except the 26-week study in *Cynomolgus* monkey. In this study, the NOAEL was set to 4 mg/kg/day based on a premature sacrifice of one male in the high dose-group administered 13.5 mg/kg 3 times/day (40 mg/kg/day) on day 85 due to persistent signs of anaemia, increased bleeding tendency, abnormalities in haematological and clinical chemistry parameters including presence of anti-FVIII antibodies. It has not been shown that the severe toxicity seen is caused by the detected antibodies. As a result of retrospective screening of the 2-week, 13-week and 26-week toxicity studies in *Cynomolgus* monkey, four additional animals were identified that developed anti-FVIII antibodies during study drug administration albeit without clinical signs. In two of these cases, the presence of anti-FVIII antibodies was found to be transient under treatment. Although bleedings are related to the primary pharmacology of caplacizumab, these events are adverse, and should not be disregarded when defining the NOAEL. Increased bleeding (dose dependent and reversible) was observed at all doses tested and a NOAEL and exposure margin to the clinic is therefore not possible to define (see pharmacology section). It should be noted that all toxicity studies were performed in healthy animals and caplacizumab related effects may differ in aTTP patients. In summary, the main finding in repeat-dose toxicity studies of caplacizumab in guinea pigs and *Cynomolgus* monkeys was an increased bleeding risk, which is related to the pharmacology of caplacizumab.

Genotoxicity

In line with ICH S6(R1), genotoxicity studies are not considered relevant for proteins and were not performed with caplacizumab.

Carcinogenicity

In line with ICH S6(R1), no carcinogenicity studies have been performed. No carcinogenic risk was identified based on the pharmacological mode of action, results from chronic dosing toxicity studies in *Cynomolgus* monkey (up to 26 weeks) or other specific characteristics of caplacizumab.

Reproduction Toxicity

The potential effects of caplacizumab on male and female fertility was assessed by evaluation of the reproductive tract (organ weights and histopathological evaluation) in the 13- and 26-week repeat dose toxicity studies in *Cynomolgus* monkey according to ICH S6(R1). The GLP-histopathological evaluation revealed no caplacizumab-related microscopic alterations in male or female reproductive organs. Male and female fertility including functional assessment were also included as part of a 13-week repeat-dose toxicity study in *Cynomolgus* monkey. At the end of the study period, male animals were evaluated for testicular size by ultrasound and for sperm function by spermiogram (both non-GLP), in addition to histopathological analysis of testis and epididymis (GLP). In female animals, in addition to histopathological analysis of the reproductive organs (GLP-compliant), oestrus cycles were monitored by analysis of vaginal cytology every 28 days, starting on day 35 (with day 0 as first day of dosing; non-GLP compliant). Based on results obtained in the 13-week repeat-dose toxicity studies in *Cynomolgus* monkey it appears that no caplacizumab related effects were observed on the male and female fertility parameters evaluated. The functional assessments on male and female fertility parameters, although conducted non-GLP, are considered well performed by qualified experts and therefore considered reliable. Further, as outlined in ICH S5(R2) histopathology of the testis has been shown to be the most sensitive method for detection of effects on spermato-genesis.

To support clinical development in women of child bearing potential a formal segment II study on embryo-foetal development was conducted in guinea pigs by i.m. administration and a follow-up TK study to assess placental transfer as well as maternal and foetal plasma levels of caplacizumab. Exposure levels of caplacizumab were similar in this study as compared to other toxicity studies following s.c. injections. In the embryo-foetal development segment II study conducted in guinea pig none of the animals died prematurely. No signs of systemic maternal toxicity were noted and no treatment-related influence was noted on the body weight, the body weight gain, the net weight change from day 6 onwards and the food and drinking water consumption. No treatment-related influence was noted on the number of corpora lutea, implantation sites, resorptions, sex distribution, foetal and placental weights, number of live foetuses at birth and the values calculated for the pre-and post-implantation loss when compared to the control. No treatment-related malformations or variations were noted during external/internal macroscopic examinations of the foetuses or soft tissue examination of the foetal heads (according to WILSON). In addition, skeletal examination (according to DAWSON) revealed no treatment related malformations, variations or retardations. The embryo-foetal toxicity study in guinea pigs showed that high and relevant maternal exposure leading to some PD response did not lead to maternal toxicity beyond some bleeding events or defects in placenta function. The placental transfer study with caplacizumab in pregnant guinea pigs qualitatively demonstrated foetal exposure to caplacizumab but exact concentrations could not be measured in the foetuses. Exposure of the dams had no effects on foetal development.

No juvenile toxicity studies were performed with caplacizumab considering that the intended clinical use is for treatment of adult patients with thrombotic thrombocytopenia.

Due to the clinical experience in pregnant vWF patients (type 1), which can serve as a model for predicting outcome of caplacizumab exposure of pregnant women or influences of caplacizumab treatment on fertility, and the lack of effects in the conducted embryo-foetal development study in guinea pigs, which did not cause concern on caplacizumab treatment during embryo- and organogenesis and later development, no pre- and postnatal development study were performed.

Toxicokinetic data

Local Tolerance

Local tolerance was studied in a dedicated study in rabbits (a non-cross-reactive species), by caplacizumab administration via the i.v., s.c., i.m., intra-arterial (i.a.) and paravenous routes at doses up to 1.2 mg/kg b.w. administered in 0.5 mL/kg (Study 20340).

Table 5: Overview of dedicated local tolerance with caplacizumab study in rabbits

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dose Levels	Gender and No. per Group	Noteworthy Findings	Study Number
rabbit, Himalayan	intravenous, intramuscular, intra-arterial, paravenous and subcutaneous (D-PBS, 0.2 M Glycine, 0.02 % Tween 80; pH 7.1 ± 0.1)	5 x 2.35 mg/ animal	6 male animals	No treatment-related macroscopic or microscopic findings were observed following administration via any of the tested routes.	LPT 20340/06

Local tolerance of caplacizumab following the clinical dose routes (intravenous and subcutaneous injection) was assessed in standard toxicity studies.

No treatment-related alterations were observed in the dedicated local tolerance study in rabbits. Local tolerance was also assessed after repeated administration as part of the 13- and 26-week repeat-dose toxicity studies in C*ynomolgus* monkey and guinea pig. In these studies, injection site oedema, induration, erythema, swelling, hematoma and/or bleeding were occasionally observed. These symptoms occurred mostly at comparable frequency in control and caplacizumab-treated animals and, except for bleeding, dose-dependency was not observed. Altogether, the local tolerance of caplacizumab in non-clinical studies was acceptable.

Other toxicity studies

Immunogenicity

The immunogenic potential of caplacizumab was evaluated in *Cynomolgus* monkey and guinea pig as part of the analysis of the pharmacokinetic behaviour and toxicokinetic evaluation of caplacizumab. In the single-dose toxicity studies in *Cynomolgus* monkey treatment emergent ADAs were observed in a few animals of the highest dose groups indicating a potential for immunogenicity. Thus, caplacizumab has low immunogenicity in guinea pigs and *Cynomolgus* monkeys.

Table 6: Summary of immunogenicity in repeat-dose toxicity studies

Study number	Species	Route	Dose (mg/kg/day)	Treatment period	Recovery period	ADA response	Day of detection	Implications
LPT 20179	Guinea pig	i.m.	0–0.05-1–20	35 days	No recovery period	No ADA detected	-	-
LPT 20095	Cynomolgus monkey	i.v.	0.0135-1.6-16	2 weeks	4 weeks	1/4 mid dose group 2/4 high dose group	17 42	No implications for toxicity assessment Both animals excluded for PK analysis – deviating PK profiles
LPT 22631	Cynomolgus monkey	i.v. s.c.	0-12 0-0.12–2.4–12	2 weeks	No recovery period	No ADA detected	-	-
LPT 24023	Guinea pig	s.c.	0-0.4-4-40	13 weeks	8 weeks	No ADA detected	-	-
LPT 24024	Cynomolgus monkey	s.c.	0-0.4-4-40	13 weeks	8 weeks	No ADA detected	-	-
Ricerca AA93337	Cynomolgus monkey	s.c.	0-0.4-4-40	26 weeks	13 weeks	1/8 low dose group 1/8 intermediate dose group 2/12 high dose group	63 202 202/244	Drug exposure affected in 1 animal (low dose) Animal excluded for toxicological evaluation and PD assessments

Day 1 is defined as first day of dosing, with exception in the 26-week chronic dose toxicity study where day 0 is first day of dosing.

2.3.4. Ecotoxicity / environmental risk assessment

Caplacizumab is a Nanobody (i.e. a protein) that is expected to undergo biodegradation into smaller peptides or amino acids which are considered non-toxic. Thus, the product is not expected to pose a risk to the environment when used according to the labelling.

2.3.5. Discussion on non-clinical aspects

Pharmacology

The non-clinical pharmacological properties of caplacizumab are considered sufficiently characterised using *in vitro* and *ex vivo* assays as well as *in vivo* models. Caplacizumab binds potently to the A1 domain of human vWF (K_D = 3.76 pM) and inhibits ristocetin-induced binding of vWF to platelets (IC_{50} = 0.26 nM) in a PD assay. Caplacizumab is cross-reactive to vWF in *Cynomolgus* monkey and guinea pig, but displays no pharmacological activity in rats, mice and dog. Thus, *Cynomolgus* monkey and guinea pigs were selected as relevant species for the toxicology testing. The safety pharmacology studies *in vivo* indicate that, besides bleeding, there are no adverse cardiovascular effects (ECG, blood pressure and heart rate) or any other findings concerning the safety parameters analysed in the repeat-dose toxicity studies in *Cynomolgus* monkey up to 40/mg/kg/day, yielding exposure margins of >100-fold to the predicted clinical exposure for the recommended dose of caplacizumab (10 mg/day). Safety endpoints (clinical signs, visual observations) were included in the repeat-dose toxicity studies in order to address respiratory function and general behavior in line with ICH S6 guideline for biotech derived products.

Pharmacokinetics

The pharmacokinetic of caplacizumab was investigated in guinea pigs and baboons as cross-reactive species, as well as in mice (not cross-reactive) following single or multiple dose administrations by the intravenous, intramuscular or subcutaneous routes. Caplacizumab toxicokinetics after repeat-dose administration was investigated in toxicology studies conducted in guinea pig and *Cynomolgus* monkey. ELISA analytical methods were developed to determine caplacizumab levels in biological samples. The analytical methods were validated and reliable across the concentration ranges tested in the studies.

After repeated administration in guinea pig and *Cynomolgus* monkey in toxicology studies, accumulation of caplacizumab was observed. The observed accumulation is consistent with expectations for the dosing regimens (3/4 times per day) that were selected to attain sufficiently high levels of exposure to define a NOAEL and adequate exposure margins for clinical trials. In the clinical situation on the other hand, a once daily dosing regimen was chosen based on pharmacodynamic considerations, i.e. to maintain a complete suppression of the RICO activity, and limited accumulation was observed.

Regarding immunogenicity, a few animals were ADA-positive during toxicity studies. A neutralising potential of the ADAs was not evaluated using a dedicated neutralisation antibody assay. Instead, the ADA results were correlated with results from PK and PD assays to evaluate any impact of ADA on caplacizumab levels and activity. In summary, two animals of the 2-week repeated dose toxicity study in *Cynomolgus* monkeys, both confirmed as ADA positive, were excluded for TK analysis because of deviating PK results (increasing caplacizumab concentrations over time) compared with the other animals, but were included for toxicity interpretation. Active exposure to drug was demonstrated through measurement of total active drug (by the use of a PK assay measuring both free and target-bound caplacizumab) and PD assessment (vWF neutralization).

Anti-drug antibodies were observed but did not affect drug exposure, with the exception of one animal in the 26-week *Cynomolgus* monkey study, which consequently was excluded from the study interpretation.

No dedicated metabolism or excretion studies were performed and this is considered acceptable and in line with ICH S6(R1). The metabolic pathways of biotechnology-derived pharmaceuticals are generally understood and include degradation to small peptides and individual amino acids.

Taken together, the pharmacokinetics of caplacizumab has been adequately characterized from a preclinical perspective.

Toxicology

The toxicity of caplacizumab has been adequately characterised in relevant non-clinical species at pharmacologically active dose levels. Active exposure to caplacizumab was demonstrated through measurement of total active drug and PD assessment (vWF neutralization). In all studies, pharmacology-related decreases in vWF:Ag and consequently FVIII:C were noted in *Cynomolgus* monkey and to a lesser extent in FVIII:C in guinea pig. Excessive pharmacology in the form of increased bleeding was identified as the only relevant safety risk. Increased bleeding (dose dependent and reversible) was observed at all doses tested and a NOAEL and exposure margin to the clinic is therefore not possible to define. It should be noted that all toxicity studies were performed in healthy animals and caplacizumab related effects may differ in aTTP patients. The local tolerance of caplacizumab in non-clinical studies was acceptable.

2.3.6. Conclusion on the non-clinical aspects

The non-clinical data supports the approval of Cablivi from a non-clinical perspective.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

2.4.2. Pharmacokinetics

Bioanalysis methods

In support of the Phase Ia clinical study ALX-0081-01/06 the concentration of caplacizumab in human plasma was determined as total active caplacizumab by an ELISA-based assay measuring both free and target-bound active caplacizumab. Detection occurred via its target (vWF) using a polyclonal rabbit anti-human vWF antibody (Ab) and before detection excess vWF was added to saturate all active nanobodies.

In all other clinical studies, the PK assay strategy was adjusted by using a target independent ELISA assay, measuring total caplacizumab. In this format human plasma samples were pre-incubated with acid to overcome target or pre-Ab interference. These samples were captured in microtiter plates coated with a mouse monoclonal anti-caplacizumab antibody and caplacizumab captured onto the coated plates was detected using a biotinylated anti-caplacizumab nanobody.

Bioanalysis of pharmacodynamic markers and ADA

The activity of the drug is also monitored using the Ristocetin Induced Platelet Aggregation (RIPA) or Ristocetin Cofactor Activity (RICO) Assay as a pharmacodynamics (PD) marker. The antibiotic Ristocetin activates vWF, which is similar to high shear blood flow conditions which causes platelet agglutination. The mechanism can be blocked by the interaction of caplacizumab with vWF. Both assays yield equivalent results but an advantage of RICO over RIPA is that the former assay may be performed using frozen plasma and lyophilized donor platelets.

Also, the level of vWF antigen in plasma samples were followed. The diagnostic immune-assay kit STA-LIATESTVWF:Ag was validated and used in the TTP PhII study ALX-0681-2.1/10 and in the PhI bioequivalence study in healthy volunteers (ALX-0681-C102). In this diagnostic assay the vWF-antigen present in test samples is captured using anti-vWF antibodies that are covalently bound to latex microparticles. The formed antigen/antibody complex results in an agglutination of the latex microparticles which is measured turbidimetrically. The von Willebrand factor antigen measurement is performed using the spiking method, in which the Owren-Koller diluent contains at least 5.0 µg/mL caplacizumab.

Anti-drug antibody (ADA) responses were detected using an ELISA based sequential bridging assay in the Phase I studies as well as in the Phase II PCI trial (ALX-0081-2.1/09). This assay could not tolerate

presence of drug in the study samples but was considered fit for purpose in support of earlier studies with short-term caplacizumab treatment.

From the Phase II TTP study (ALX-0681-2.1/10) an improved drug tolerance assay was implemented which was also used in support of the PhI bioequivalence study in healthy volunteers (ALX-0681-C102). The homogenous bridging assay utilises a Meso Scale Discovery (MSD) platform. In this assay format human serum samples possibly containing anti-caplacizumab antibodies were pre-incubated overnight with an excess of biotinylated and sulpho-tagged caplacizumab. The formed immune complexes were captured on a streptavidine coated MSD plate and detected via electrochemiluminescence (ECL).

ADA positive subjects included in the TTP PhII study ALX-0681-2.1/10 were further characterized in an additional assay to differentiate pre-Ab from treatment emerging (TE) ADA. This assay (mADA) is a modification of the homogenous bridging assay on the MSD platform, by using a C-terminal alanine extended analogue of caplacizumab (addition of one alanine) as the sulpho tagged detection tool (sulfo tagged ALX-0081-ala). The mADA assay predominantly measures TE ADA, as pre-Ab will not or to a lesser extent bridge in this assay format. Only TE ADA responses restricted to the C-terminal region in the Nanobody will be left undetected using the mADA assay and cannot be differentiated from pre-Ab.

Data analysis

A population PK/PD model, characterising the relationship between drug exposure and the pharmacodynamics effect (in terms of total vWF levels), was built using nonlinear mixed-effects modelling.

Data was included from Study 1 (ALX-0081/0681-01/06), Study 2 (ALX-0081/0681-1.2/08a) and Study 3 (ALX-0081/0681-1.2/08b), Study 4 (ALX-0081/0681-1.2/08c OLE), Study 5 (ALX-0081/0681-1.1/08 First part) and Study 6 (ALX-0081/0681-1.1/08 Second part), Study 7 (ALX-0081/0681-2.1/09), Study 8 (ALX-0681-2.1/10), Study 9 (ALX-0681-C102) and Study 10 (ALX-0681-C301).

The caplacizumab-vWF pkpd model included a two-compartment drug disposition model with first-order linear elimination of free drug and two parallel first-order absorption processes following s.c. dosing of caplacizumab. The model described the formation of a drug-vWF complexes with the ability to both form dimer and trimers. The production, maturation and release of vWF were described by transit compartments and a vWF pool with feedback effects stimulating the production and release of vWF when vWF decrease below the subjects baseline level. For aTTP patients, disease progression and effects governed by PE treatment were adequately captured by i) a disease progression model dependent on time and ii) removal of free vWF, free drug and drug-vWF complex by PE.

Absorption

The proposed dosing schedule of caplacizumab includes an intravenous dose on day 1 and following days the drug is administered subcutaneously. The absolute bioavailability of caplacizumab was estimated to 90.1% in the population pkpd analysis.

Bioequivalence

A single centre, open-label, randomized, single dose cross-over bioequivalence study to compare the reconstituted lyophilised formulation (to be marketed) with the reference liquid formulation of caplacizumab. 24 healthy subjects were included, a total dose of 10 mg was administered s c, and PK/PD sampling was performed for 6 days. The total washout time between doses was 14 days.

Bioequivalence was shown both for Cmax (ratio 97.2 , 90% CI 93.8-100.8) and AUC0-∞ (ratio 102.4, 90% CI 97.8-107.3).

PD parameters RICO and vWF:Ag were also measured at the same timepoints. The results were similar between treatments. A statistically significant difference in onset of complete RICO inhibition was observed, where median time to onset for treatment A was 2 hours and treatment B was 4 hours. The duration of RICO inhibition as well as tmax/Emax for vWF:Ag were however the same.

After subcutaneous administration, caplacizumab is rapidly and almost completely absorbed (estimated F> 0.901) in the systemic circulation.

Distribution

After absorption, caplacizumab binds to the target and distributes to well perfused organs. In patients with aTTP the central volume of distribution was estimated at 6.33 L.

Elimination

Caplacizumab is subject to target-mediated drug disposition meaning that its pharmacokinetics (PK) is influenced by the binding to vWF. It is also subject to non-target mediated elimination which may be a combination of catabolism and renal elimination.

No elimination studies have been performed. For a protein of this size, renal filtration followed by metabolism in the kidney is expected for the free drug. The target bound drug is expected to be hepatically cleared as its complex with vWF (se PKPD model).

Caplacizumab half-life is concentration- and target level dependent.

Special populations

Renal impairment

Caplacizumab has a molecular weight of 28 kDa, which is below a commonly accepted threshold of 70 kDa for glomerular filtration. Thus, caplacizumab is expected to be subject to glomerular filtration. The effect of impaired renal function on caplacizumab pharmacokinetics has not been formally studied in the TTP population. The impact of reduced renal function (as measured by creatinine clearance, CRCL) on the linear clearance of caplacizumab was tested in the pkpd model. The effect was statistically significant but numerically small; the effect of CRCL was implemented as a power model and estimated to 0.15.

Hepatic impairment

No formal study of the impact of hepatic impairment on the PK of caplacizumab has been performed. Caplacizumab, free or bound, is expected to be subject to protein catabolism and liver impairment in itself is not expected to have an impact on this catabolism.

Gender

In the dataset for the pkpd model, 41.4% of the subjects were female. The pk of caplacizumab was not seen to be different between male and female subjects.

Race

The majority (89.5%) of the subjects in the PKPD dataset were Caucasian. No definitive conclusions can be drawn regarding the impact of race on the pk of caplacizumab. A slight difference could be

detected in the baseline value of VWF:Ag comparing Caucasian subjects to Black subjects. This difference is not expected to influence the response to caplacizumab treatment.

Weight

In the pooled population PK data set body weight ranged from 46.5 kg to 150 kg with a median value of 80 kg. This effect of body weight on caplacizumab PK was included in the population PK model using allometric scaling of disposition parameters with fixed exponents. The effect of body weight on the pk of caplacizumab was limited and resulting in an even smaller difference in pharmacodynamic response.

Age

The median age (min – max) in the pkpd data set was 52 years (18 years – 85 years). Based on literature data (Albnez S, Ogiwara K, Michels A, Hopman W, Grabell J, James P and Lillicrap D, 2016, Aging and ABO blood type influence von Willebrand factor and factor VIII levels through interrelated mechanisms. *Journal of thrombosis and haemostasis*: JTH vol. 14: 953–963). In Healthy volunteers and PCI patients, age was included as a covariate on the complex elimination rate constant (Kcom) with younger patients (<40 years) having 50% faster elimination. The effects of age identified in healthy volunteers and PCI patients are not considered of any particular concern, and were not confirmed in the aTTP patient population.

ADA

Only 6 aTTP patients showed evidence of treatment-emerging anti-drug antibodies (ADA). No effect of ADA was seen in the updated population PKPD analysis. Individual observed data did not reveal any major impact on the PK or PD of caplacizumab.

Pharmacokinetic interaction studies

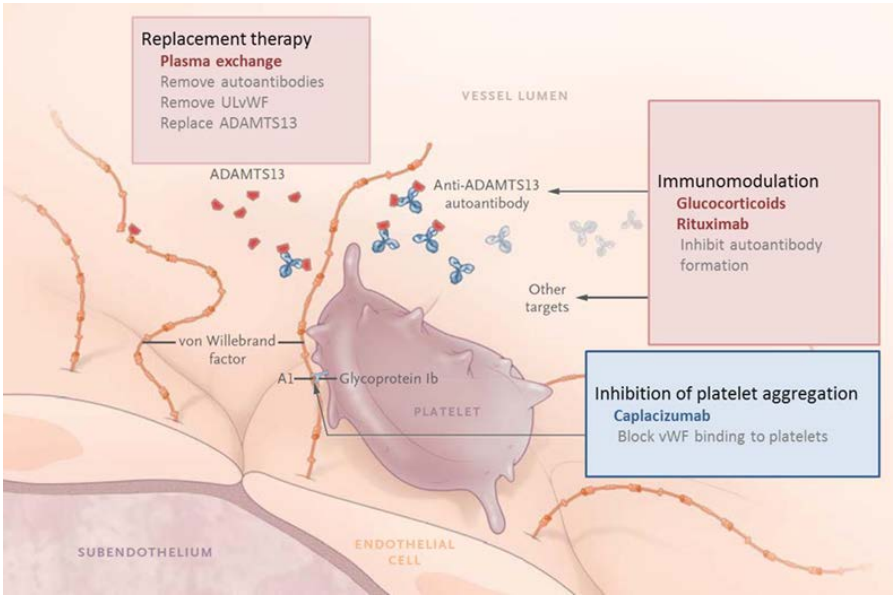
No *in vitro* or *in vivo* DDI studies have been performed.

2.4.3. Pharmacodynamics

Mechanism of action

The normal processing of vWF from ultralarge multimers to normal sized multimers is blocked by inhibitory antibodies against the proteolytic enzyme ADAMTS13 in aTTP. This will lead to the formation of ULvWF-mediated microthrombi. Current standard of care consists of immunosuppressants which will block the formation of these antibodies, and PE which has a triple action of (i) removing ULvWF and introducing donor vWF, (ii) removing inhibitory antibodies against ADAMTS13 and (ii) replenishing active ADAMTS13 from the donor plasma. Caplacizumab blocks the ULvWF-mediated platelet interaction, which can be measured through a PD marker for vWF neutralisation (vWF:RICO), see figure below.

Figure 3: Diagram of Mechanism of action of caplacizumab and standard of care in aTTP.



Primary and Secondary pharmacology

Pharmacodynamic markers in clinical studies

In study **ALX-081-01/06** the RIPA assay was used to measure the neutralisation of vWF by caplacizumab. Full inhibition of RIPA was defined as maximal aggregation <10%.

In all placebo-treated subjects, except in subject 1002 from the first cohort, the RIPA results remained unchanged and were within the normal variation of the assay. In the first 2 cohorts, no consistent decrease of RIPA was observed for all subjects dosed with caplacizumab. From cohort 3 (4 mg) onwards, all subjects receiving caplacizumab showed an inhibition to values below 10%, which occurred directly at the end of the infusion at 1h post, lasting from 1 to 6 h after start of infusion.

In subjects treated with caplacizumab, transient decreases in vWF:Ag levels were noted after dosing. These decreases reached a maximum between 6h and 12h post-dose and ranged from about 20% to 40% of baseline. vWF:Ag levels recovered to baseline levels from 24h to 48h after dosing. In the placebo treated subjects, no substantial changes in vWF:Ag levels were noted during the study. No dose dependency could be assigned to these changes in vWF:Ag.

In **study ALX-0681-1.1/08**, RICO was used as a measurement of vWF activity, and a RICO activity of <20% reflected complete inhibition of vWF to bind platelets.

In the placebo treated subjects, no changes in RICO were noticed, while in all treatment groups of the caplacizumab treated subjects, a rapid decrease from baseline could be found. Decreases below the threshold for complete RICO inhibition (<20%) were noted in all treatment groups and were reversible. In the dose groups of the SAD part, complete RICO suppression was observed from 4 to 12h after dosing and returned to baseline after approximately 72 h after dosing. Similarly for both the MD groups, a rapid and sustained decrease below the threshold for complete RICO inhibition could be observed which pertained from Day 1 to 8 (7-day MD group) and Day 1 to16 (14- day MD group).

Levels were back at baseline for the follow up measurement at Day 15 and 22 for the 7-day and 14-day MD group.

In **study ALX-0681-2.1/10**, all patients received 10 mg daily doses of caplacizumab and RICO was used as the measure of vWF activity.

At Baseline, the mean RICO activity was approximately 80% in both treatment groups. In the caplacizumab treatment group, the mean RICO activity was reduced to levels close to or below 20% by Day 1 of daily PE. Mean RICO activity remained mostly < 20% throughout the first week of the daily PE treatment period, with the exception of some subjects with treatment interruption or withdrawal. Mean RICO activity was as well < 20% throughout the post-daily PE period. By Day 3 of the follow-up period, mean RICO activity in the caplacizumab treatment group had increased to approximately 40%. By Day 7 of the follow-up period, mean RICO activity had returned to Baseline levels in the caplacizumab treatment group.

Mean vWF:Ag levels were higher at baseline compared to the mean vWF:Ag levels observed in healthy subjects (180% and 190% in the caplacizumab and placebo treated group respectively). In the caplacizumab treatment group, there was a decrease in mean vWF:Ag levels by Day 1 of daily PE. The mean vWF:Ag levels decreased further by Day 2 of daily PE to about 100% and remained at similar levels until the first day after daily PE. Thereafter, vWF:Ag levels increased slightly during the post-daily PE period, but remained well below Baseline levels throughout dosing. After caplacizumab treatment was stopped, vWF:Ag levels quickly returned to Baseline levels by Day 7 of follow-up. In the placebo treatment group, after an initial slight decrease, vWF:Ag levels increased to above Baseline levels and remained at above or around Baseline until the end of the post daily PE period and finally decreased to about 150% at the follow up period.

Only one dose (10 mg daily) was tested in aTTP patients or in the multiple-dose setting in healthy volunteers. The Applicant provides a discussion regarding the choice of dose, based on expected exposure and corresponding effect on total vWF:Ag levels for different dose levels, simulated from the PKPD model. The model predicted that target drug levels ensuring the desired pharmacodynamics effect can be attained with the proposed dosing regimen, but not with a lower dose of 5 mg per day. A higher dose of 15 mg od would not substantially increase efficacy.

2.4.4. Discussion on clinical pharmacology

In general the bioanalysis methods in use to support the PK and PD in the clinical studies are adequately validated.

The additional characterisation method in use to distinguish between pre-existing antibodies (Pre-Ab) and treatment emerging (TE) ADA was not satisfactory to detect TE ADA in subjects classified as equivocal (pre-Ab levels above a defined threshold that can defy detection of TE ADA). However, a strategy is in place to take into consideration the post-dose ADA signals for the data interpretation which is acceptable.

In the SAD and MAD studies in healthy volunteers, the AUC increased less than dose proportionally. This can be interpreted as a saturation of the target with higher dose, and a more rapid clearance of excess free caplacizumab. Caplacizumab is subject to target-mediated drug disposition meaning that its pharmacokinetics (PK) is influenced by the pharmacodynamics (PD). Due to the complex PK and PD relationships, the use of modelling and simulation to support the characterisation of caplacizumab PK and the pharmacodynamic effects was used. The model was considered to describe the data and perform simulations within the studied range of doses, treatment period, covariate values and other determinants of the model. The model provides support for the selected dosing regimen.

The bioequivalence study has shown bioequivalence in terms of PK between the solution formulation and the lyophilised formulation to be marketed. The minor PD difference seen regarding RICO inhibition is not considered clinically relevant. The population PKPD model also support bioequivalence between the two formulations.

The Applicant included the effect of body weight on the non-target mediated clearance of caplacizumab in the population model using allometric scaling. Simulations show that the influence of body weight on the exposure to caplacizumab and pharmacodynamic response was limited.

No elimination studies have been performed. This is acceptable. For a protein of this size, renal filtration followed by metabolism in the kidney is expected for free drug. Even though reduced renal function was found to have a limited effect on the disposition of caplacizumab, the Applicant explored the issue by simulating the effect of a substantial decrease in the linear non-target mediated clearance. The effect on caplacizumab exposure was still limited and did not impact the pharmacodynamic response according to the pkpd model. The target bound drug is expected to be hepatically cleared as its complex with vWF.

The Applicant looked at the impact of ADA on the individual PK and PD data for the 6 aTTP patients that developed ADA during treatment and compare to the whole population. There was no sign of deviation from the PK and PD compared to the total population.

No *in vitro* or *in vivo* DDI data have been provided. No classical pharmacokinetic interactions are expected for this type of protein. It is also agreed that the vWF-pathway is not likely to regulate CYP enzyme expression. The lack of data is thus acceptable. The TTP patients are co-medicated with immunosuppressants, which likely modulate the disease.

The PD data collected on vWF activity (RICO/RIPA) from healthy volunteers as well as TTP patients confirms the pharmacological effect and that 10 mg is an active dose. A modelling exercise was used to confirm that the proposed 10 mg od dosing regimen is adequate.

2.4.5. Conclusions on clinical pharmacology

The pharmacokinetics (PK) and pharmacodynamics (PD) of caplacizumab have been characterised in healthy volunteers and in aTTP patients.

2.5. Clinical efficacy

2.5.1. Dose response and main studies

Table 7: A summary of clinical studies in aTTP

Study (EudraCT number)	Clinical Phase	Design	Study Population	Treatment	Objectives
ALX-0681-2.1/10 "TITAN" (2010-019375-30)	II	Single-blind, randomized, parallel-group, placebo-controlled, multi-center	Patients with aTTP n=75 (randomized) n*=72 (treated)	Treatment in addition to standard of care. Caplacizumab group (n=36, n*=35): 10 mg i.v. prior to first PE on-study, followed by 10 mg s.c. after each PE session. Daily s.c. administration of 10 mg study drug for a period of 30 days after last PE. Placebo group (n=39, n*=37): administration as per caplacizumab.	Primary: To demonstrate a reduction in the time to a confirmed platelet response. Secondary: To evaluate various other disease-related endpoints, plus safety, immunogenicity, PK and PD.
ALX0681-C301 "HERCULES" (2015-001098-42)	III	Double-blind, randomized, parallel group, multicenter placebo-controlled	Patients with aTTP n=132 (planned)	Treatment adjunctive to standard of care. Caplacizumab group (n=66 planned): 10 mg i.v. prior to first PE on-study, followed by 10 mg s.c. after each daily PE. Daily s.c. administration of 10 mg study drug for a period of 30 days after stop of daily PE. Treatment extension beyond 30 days for a maximum of 28 days guided by risk factors for relapse, including ADAMTS13 activity, signs and symptoms of continued disease activity. Placebo group (n=66 planned): administration as per caplacizumab.	Primary: to evaluate efficacy of caplacizumab in more rapidly restoring normal platelet counts as measure of prevention of further microvascular thrombosis. Key Secondary: to evaluate a composite endpoint consisting of TTP-related mortality, recurrence of TTP and major thromboembolic events during study drug treatment, and to evaluate prevention of recurrence of TTP, refractoriness to treatment and biomarkers of organ damage

Study (EudraCT number)	Clinical Phase	Design	Study Population	Treatment	Objectives
ALX0681-C302 (2016-001503-23)	IIIb	Open label, multicenter	Prospective follow-up study for patients who completed HERCULES Follow-up period of 3 years	In case of aTTP recurrence during the follow-up study: Open-label caplacizumab treatment adjunctive to standard of care: caplacizumab10 mg i.v. prior to first PE on-study, followed by 10 mg s.c. after each daily PE. Daily s.c. administration of 10 mg study drug for a period of 30 days after stop of daily PE. Treatment extension beyond 30 days for a maximum of 28 days guided by risk factors for relapse, including ADAMTS13 activity, signs and symptoms of continued disease activity	To evaluate long-term safety and efficacy of caplacizumab and the safety and efficacy .of repeated use of caplacizumab, and to characterize the long term impact of TTP

Study ALX-0681-2.1/10: A Phase II, single-blind, randomised, placebo-controlled trial to study the efficacy and safety of anti-von Willebrand factor Nanobody administered as adjunctive treatment to patients with acquired thrombotic thrombocytopenic purpura.

Methods

Study Participants

Key inclusion criteria

- Subject with a clinical diagnosis of TTP.
- Subject requiring PE (one single PE session prior to randomisation into the study was allowed).
- Willing to accept an acceptable contraceptive regimen.

Key exclusion criteria

Platelet count ≥ 100,000/µL; Severe active infection indicated by sepsis (requirement for pressors with or without positive blood cultures); Clinical evidence of enteric infection with *Escherichia coli 0157* or related organism; Anti-phospholipid syndrome; Diagnosis of disseminated intravascular coagulation (DIC); Pregnancy or breast-feeding; Hematopoietic stem cell or bone marrow transplantation-associated thrombotic microangiopathy; Known congenital TTP; Active bleeding or high risk of bleeding;

Uncontrolled arterial hypertension; Ongoing chronic treatment with anticoagulant treatment that could not be stopped safely; Severe liver impairment or severe chronic renal impairment; Known hypersensitivity to the active substance or to excipients of the study drug; Severe or life threatening clinical condition other than TTP; Use of another investigational drug or device within 30 days prior to screening.

Treatments

Study drug

The first study drug administration was as an i.v. bolus within 6 hours to 15 minutes prior to initiation of the first on-study PE or, after implementation of Protocol Version 12.0, the second PE session (if the subject was randomised after a single PE session). The first on-study PE was followed by s.c. administration of study drug within 30 minutes after the end of the PE procedure. Subsequently, daily s.c. administrations of 10 mg caplacizumab or placebo in adult subjects followed each PE session for the duration of PE (including tapering and PE given for exacerbations) and once daily for 30 days following the last PE, including tapering. If 2 PE sessions were scheduled per day, study drug was administered within 30 minutes after the end of the PE procedure resulting in a daily dose of 20 mg. Study drug administration continued in case of re-initiation of PE for an exacerbation of TTP with a maximum total treatment duration limited to 90 days after first administration of study drug. The "study drug post- PE" period (period of 30 days after the very last PE; see further below) re-commenced once PE that was reinitiated due to the exacerbation was again stopped. Re-initiation of study drug treatment in case of TTP relapse (defined as de novo event of TTP that occurred later than 30 days after the last daily PE) was not permitted. Tapering prolonged study drug administration and delayed the start of the "study drug post-PE" period. It was therefore possible that a relapse could occur ≥ 30 days after the last daily PE, but before the end of the 30-day period of study drug administration post-PE. In this case, the study drug was to be discontinued at the restart of the PE treatment.

Selection of dose

The initial i.v. administration of study drug prior to the first PE on study was justified based upon a Phase I study in healthy subjects, as well as a Phase Ib study in acute coronary syndrome (ACS) patients. In both studies, an immediate complete target neutralisation was observed, namely RIPA ≤ 10% in healthy subjects and RICO ≤ 20% in ACS patients treated with the biologically effective dose. For the Phase I study in healthy subjects, minimal effective dose was 2 mg and saturation of the effect was achieved with the highest dose of 12 mg. For the Phase Ib study, a single dose of 6 mg, followed by 3 subsequent doses of 4 mg every 6 hours, given as i.v. bolus injections was established to be the biological effective dose. It was anticipated that all active ULvWF present in the blood that has not yet aggregated platelets can be inhibited from further platelet aggregation by saturating it with the immediate i.v. injection of 10 mg anti-vWF Nanobody. This allowed for the predicted protection by the investigational product against UL-vWF mediated microthrombus formation until the time of start of PE therapy.

The further administrations of the investigational drug were performed by the s.c. injection route. The proposed s.c. dosing regimen of the caplacizumab was investigated in a Phase I study with healthy subjects. The daily dosing of caplacizumab 10 mg as s.c. injection resulted in a complete and sustainable inhibition of the biomarker RICO, indicating the complete suppression of vWF-mediated platelet adhesion for 24 hours.

Dose modification due to clinically relevant bleeding (TEAE)

Clinically relevant bleeding was the main potential risk based on the pharmacological action of caplacizumab . It was defined as moderate to severe (including life-threatening) bleeding requiring urgent medical and/or surgical intervention. In case of clinically relevant bleeding, appropriate treatment for bleeding according to standard practice was to be initiated and treatment with study drug was to be interrupted. In addition, plasma levels of vWF:Ag and FVIII:C were determined. If FVIII:C levels were < 10% the presence of anti-FVIII antibodies was to be assessed, and if presence was confirmed, study drug was to be permanently discontinued. If either or both vWF:Ag and FVIII:C were at clinically significant low levels as judged by the treating physician, administration of vWF and FVIII through commercially available combination preparations, such as Haemate-P or equivalent antihaemophilic factor/vWF complex, was to be initiated and continued until the bleeding stopped. Study drug could only be restarted when the bleeding had stopped and vWF:Ag >50% and FVIII:C levels were within normal range as per local lab ranges. The PE treatment, if applicable, was to be continued as clinically indicated.

Standard of care

Subjects received the standard of care and treatment judged appropriate by the Investigator at each site and according to site guidelines for treatment of TTP. The principal treatment for acquired TTP was daily PE. Local practice differs with regard to the frequency of PE being occasionally reduced ("tapered") rather than stopped completely at time of response which was at the discretion of the Investigator, though not recommended per this protocol.

Additional treatment could include one or more of the following, which was also variable depending on local standard practice: adjunctive immunosuppressive treatment (e.g., corticosteroids, rituximab), antiplatelet agents (e.g., aspirin), supportive therapy with red cell transfusion or folate supplementation and treatment with vincristine or cyclosporin in case of refractory TTP. After platelet counts had partially recovered, LMWH could be used prophylactically in subjects at high risk of venous thromboembolism. In this case heparin was administered according to local institutional guidelines, or in the absence of these, after a platelet count of ≥ 100,000/µL had been reached.

Prior therapy

Upon inclusion in the study, chronic treatment with anticoagulant treatment such as vitamin K antagonists, heparin (or LMWH) and non-acetyl salicylic acid non-steroidal anti-inflammatory molecules was discontinued. If recommended by local guidelines, aspirin could be continued. Heparin could be administered according to local institutional guidelines, or in the absence of these, after a platelet count of ≥ 100,000/µL had been reached. Study drug administration continued in case of re-initiation of PE for an exacerbation of TTP with a maximum total treatment duration limited to 90 days after first administration of study drug. Re-initiation of study drug treatment in case of TTP relapse (defined as de novo event of TTP that occurred later than 30 days after the last daily PE) was not permitted

Objectives

Outcomes/endpoints

Primary endpoint

Time to recovery of platelets (≥ 150,000/µL), confirmed at 48 hours after the initial reporting of platelet recovery by a *de novo* measure of platelets ≥ 150,000/µL and LDH ≤ 2 x ULN. Observations were censored at 30 days after first administration of study drug.

Main secondary endpoints

- Number and percentage of subjects with complete remission (defined as confirmed platelet response and absence of exacerbation for 30 days after the last daily PE);
- Number and percentage of (subjects with) exacerbations of aTTP (defined as recurrent thrombocytopenia following a confirmed platelet response and requiring a re-initiation of daily PE treatment between ≥ 1 day but ≤ 30 days after the last daily PE) and time to first exacerbation of aTTP. However, due to a discrepancy between the protocol and the statistical analysis plan (SAP), this analysis was reported through up to 12-months of follow-up (as described in the SAP) with a post-hoc analysis of all exacerbations/relapses reported through up to 1 month of follow-up;
- Number and percentage of subjects relapsing with aTTP (defined as *de novo* event of aTTP that occurs later than 30 days after the last daily PE);
- Total mortality within the daily PE treatment period and within the subsequent study drug treatment period (including tapering).

In addition, in line with the amended protocol version 12.0, a stratified analysis based on the presence (n=6) or absence of a prior PE before randomization was performed for all primary and secondary efficacy endpoints.

Sample size

Originally, the study was to enroll 110 subjects in a 1:1 allocation to caplacizumab or placebo. This sample size was based on a log-rank test with a power of 80% at a one-sided 2.5% significance level to detect a 44% reduction in median time-to-platelet response (3.4 days on caplacizumab compared to 6 days on placebo). The sample size also incorporated an assumption that 15% of subjects would be lost-to-follow-up.

The study was open for enrolment from October 2010 to January 2014, when recruitment was halted because of persistent recruitment challenges in this orphan disease setting. Seventy-five patients were randomized: 36 to the caplacizumab group and 39 to the placebo group.

Randomisation

Adult subjects were assigned to one of the two treatments via an interactive web-based system according to a computerised randomisation schedule. Adolescent subjects were not to be randomised and were to receive only caplacizumab. No adolescents were treated during the study.

When the randomisation number and treatment assignment were obtained, the randomisation number was recorded on the case report form (CRF). As this was a single-blinded study, the Investigator was informed of the treatment at the time of randomisation.

Blinding (masking)

This is a single-blinded study.

Statistical methods

In addition to the Protocol, a description of the statistical methods applied in this study was given in the SAP (dated 09-Dec-2013). The final SAP was finalised before the database lock on 23-May-2014.

All hypothesis testing was 2-sided unless otherwise specified and carried out at the 5% significance level and designed to evaluate the superiority of caplacizumab

to placebo. No adjustment for multiplicity was applied to the analysis for the secondary variables. The primary, secondary and longer term endpoint analyses were based on available data. No imputations were made for missing data unless otherwise specified.

Primary Efficacy Analysis

The primary efficacy endpoint, time-to-platelet response, was measured in days, hours and minutes from the time of first study drug administration. A KM analysis with time-to-response as the endpoint and treatment group as the independent variable was performed on the ITT and Per Protocol (PP) populations. An analysis was performed for all subjects and for subjects stratified for absence/presence of one PE session prior to randomization. If the response was not reached within 30 days after first administration of study drug or data were not available for any reason (e.g. death, withdrawal, loss-to-follow-up), then the endpoint was censored for that subject. Caplacizumab was compared with placebo using a 1-sided log-rank test in order to assess superiority at 2.5% significance level.

In addition to the KM estimates, the corresponding Cox proportional hazard regression model with Baseline disease characteristics (ADAMTS13 activity < 5% versus ≥ 5%, vWF:Ag level [continuous], first episode versus recurrent disease, presence or absence of RICO suppression of < 20% throughout treatment period, one PE prior to randomisation or not) was used to estimate the hazard ratio (HR) and associated 95% CIs for the HR for caplacizumab and the placebo group. This was represented by means of Forest plots.

The time to first exacerbation of aTTP, first relapse of aTTP, and first exacerbation or relapse from first study drug administration, followed the same analysis method as the primary efficacy endpoint and was presented graphically.

Sensitivity analyses consisted of analyses performed on the PP Population for the primary efficacy endpoint; analysis of the treatment effect adjusted for various covariates, such as ADAMTS13 activity at Baseline; analysis of non-confirmed and confirmed platelet response; assessment of impact of the use of rituximab during daily PE.

Interim Analysis

On 13-Jan-2014, the decision to stop subject recruitment into the study due to the low recruitment rate was communicated to the study sites. It was decided that the conduct of the study would be stopped when the last subject randomised reached the 1-month follow-up visit. The decision was not for any reason of safety; it was also not based on any analysis of data, neither formal nor informal, but was based on strategic considerations for further investment which required having the study data fully and formally analysed.

An interim analysis for safety with formal stopping rules was performed when 28 of the caplacizumab treated subjects had been treated and assessed. The DSMB reviewed the safety data collected from

the first 56 subjects (evenly split between caplacizumab and placebo) enrolled and treated in the study, with an ensuing follow-up of 30 days. On 31-Jan-2014, the recommendation of the DSMB to continue the study with no changes to the Protocol based on the interim analysis results was communicated to the Sponsor. Since no efficacy analyses were included in the interim analysis, no alpha adjustment was made.

Results

Participant flow

Subject disposition and analysis populations (all subjects)

	Caplacizumab	Placebo	Total
Screened	NA	NA	76
Screen failures			1
Randomized	36	39	75
Not treated	1 (2.8%)	2 (5.1%)	3 (4.0%)
Safety Population	35 (97.2%)	37 (94.9%)	72 (96.0%)
ITT Population	36 (100%)	39 (100%)	75 (100%)
PP Population	10 (27.8%)	15 (38.5%)	25 (33.3%)
PK Population	35 (97.2%)	1 (2.6%) [1]	36 (48.0%)
Attended 1-month follow-up visit	32 (88.9%)	31 (79.5%)	63 (84.0%)
Attended 3-month follow-up visit	27 (75.0%)	25 (64.1%)	52 (69.3%)
Attended 6-month follow-up visit	25 (69.4%)	23 (59.0%)	48 (64.0%)
Attended 12-month follow-up visit	22 (61.1%)	21 (53.8%)	42 (56.0%)
Completed study	20 (55.6%) [2]	21 (53.8%)	41 (54.7%)
Discontinued prematurely	16 (44.4%)	18 (46.2%)	34 (45.3%)
If discontinued, primary reason:			
Adverse event/drug reaction	3 (8.3%)	0	3 (4.0%)
Subject withdrew consent	1 (2.8%)	3 (7.7%)	4 (5.3%)
Lost to follow-up	1 (2.8%)	0	1 (1.3%)
Physician decision	1 (2.8%)	1 (2.6%)	2 (2.7%)
Protocol violation:	0	1 (2.6%)	1 (1.3%)
Non-compliance with study drug	0	0	0
Non-compliance with visit schedule	0	0	0
Treatment with prohibited medication	0	0	0
Other	0	1 (2.6%)	1 (1.3%)
Study terminated by sponsor	9 (25.0%)	10 (25.6%)	19 (25.3%)
Pregnancy	0	1 (2.6%)	1 (1.3%)
Death	0	1 (2.6%)	1 (1.3%)
Other	1 (2.8%)	1 (2.6%)	2 (2.7%)

Recruitment

Date of first enrolment: 7 Jan 2011; Date of last completed: 14 Mar 2014.

32 active sites (out of 56 approved sites) in 11 countries: Australia (1 site), Austria (1 site), Belgium (4 sites), France (1 site), Germany (5 sites), Israel (2 sites), Italy (4 sites), Spain (3 sites), Switzerland (2 sites), United Kingdom (UK; 1 site) and the United States of America (USA; 8 sites).

Note that the study was prematurely terminated due to recruitment challenges: 75 adult subjects (36 to the experimental arm and 39 to placebo) were enrolled while 110 were planned in accordance with the sample size estimation.

Conduct of the study

The study recruitment was stopped before the planned number of patients was included due to the low recruitment rate. Seventy five patients were randomised into the study without follow-up.

Several amendments to the protocol were done during the course of the study. For example the primary endpoint was changed before inclusion of the first patient. The inclusion- and exclusion criteria, the significance level, the definition of the ITT population and the number of patients were changed. Furthermore, several post-hoc analyses were performed that were neither described in the protocol nor in the SAP. A relatively large fraction of major protocol violations was reported as well as issues related to local and central laboratories, leading to issues with the sample/data integrity. In addition, fractions of missing data are for certain analyses large and sometimes unknown.

Baseline data

Summary of subject demographics (ITT population)

		Caplacizumab N=36	Placebo N=39	Total N=75
Age (years) [1]	n	36	39	75
	Mean	40.6	42.5	41.6
	SD	12.70	13.18	12.90
	Median	39.5	41.0	40.0
	Min, Max	19, 72	21, 67	19, 72
Gender	Male	12 (33.3%)	19 (48.7%)	31 (41.3%)
	Female	24 (66.7%)	20 (51.3%)	44 (58.7%)
Ethnicity / Race	Caucasian	32 (88.9%)	34 (87.2%)	66 (88.0%)
	Black	4 (11.1%)	5 (12.8%)	9 (12.0%)
	Asian	0	0	0
	Unknown	0	0	0

		Caplacizumab N=36	Placebo N=39	Total N=75
	Other	0	0	0
Baseline BMI (kg/m^2)	n	25	37	62
	Mean	28.70	29.33	29.08
	SD	9.111	6.690	7.692
	Median	27.76	27.76	27.76
	Min, Max	16.2, 50.7	19.4, 45.7	16.2, 50.7

Summary of baseline disease characteristics (ITT population)

[1]		Caplacizumab N=36	Placebo N=39	Total N=75
Platelets ($10^3/mm^3$)	n	35	37	72
	Mean	21.1	28.0	24.6
	SD	18.15	19.97	19.29
	Median	16.0	21.0	19.0
	Min, Max	2, 70	5, 84	2, 84
LDH >ULN (post-hoc analysis)	n (%)	32 (88.9)	32 (82.1)	64 (85.3)
ADAMTS13 activity				
< 5%	n (%)	21 (58.3)	22 (56.4)	43 (57.3)
≥ 5%	n (%)	9 (25.0)	14 (35.9)	23 (30.7)
Missing	n (%)	6 (16.7)	3 (7.7)	9 (12.0)
< 10% (post-hoc analysis)	n (%)	28 (77.8)	30 (76.9)	58 (77.3)
≥ 10% (post-hoc analysis)	n (%)	2 (5.6)	6 (15.4)	8 (10.7)
Missing (post-hoc analysis)	n (%)	6 (16.7)	3 (7.7)	9 (12.0)
ADAMTS13 Functional Inhibitors (BU/mL) [2]:				
< 0.5	n (%)	6 (16.7)	6 (15.4)	12 (16.0)
≥ 0.5 and ≤ 2	n (%)	15 (41.7)	9 (23.1)	24 (32.0)
> 2	n (%)	5 (13.9)	8 (20.5)	13 (17.3)

[1]		Caplacizumab N=36	Placebo N=39	Total N=75
>> 2	n (%)	4 (11.1)	7 (17.9)	11 (14.7)
Missing or not tested	n (%)	6 (16.7)	9 (23.1)	15 (20.0)
vWF:Ag (%)	n	23	27	50
	Mean	180.26	189.60	185.30
	SD	78.176	74.265	75.449
	Median	158.00	172.30	164.75
	Min, Max	99.0, 420.0	107.9, 434.0	99.0, 434.0
Initial Episode or Recurrent [3]:				
Initial	n (%)	24 (66.7)	27 (69.2)	51 (68.0)
Recurrent	n (%)	12 (33.3)	12 (30.8)	24 (32.0)
PE Prior to Randomization:				
Yes	n (%)	2 (5.6)	4 (10.3)	6 (8.0)
No	n (%)	34 (94.4)	35 (89.7)	69 (92.0)
Cardiac Markers:				
Troponin T or I >ULN (post-hoc analysis)	n (%)	19 (52.8%)	17 (43.6%)	36 (48.0%)

Summary of Medical History Reported as Underlying Cause of Thrombotic Thrombocytopenic Purpura (Safety Population)

	ALX-0081 N=35 n (%)	Placebo N=37 n (%)	Total N=72 n (%)
Number of subjects with abnormal medical history	11 (31.4)	15 (40.5)	26 (36.1)
Body System			
Dermatologic	3 (8.6)	1 (2.7)	4 (5.6)
HEENT	2 (5.7)	0	2 (2.8)
Respiratory	1 (2.9)	1 (2.7)	2 (2.8)
Cardiovascular	0	1 (2.7)	1 (1.4)
Genitourinary/Reproductive	2 (5.7)	2 (5.4)	4 (5.6)
Musculoskeletal	0	1 (2.7)	1 (1.4)
Neurological	1 (2.9)	8 (21.6)	9 (12.5)
Psychological/Psychiatric	1 (2.9)	1 (2.7)	2 (2.8)
Gastrointestinal	3 (8.6)	2 (5.4)	5 (6.9)
Haematologic	8 (22.9)	9 (24.3)	17 (23.6)
Other	4 (11.4)	5 (13.5)	9 (12.5)

%=percentage based on N; HEENT=Head, Eyes, Ears, Nose and Throat; N=number of subjects in the population of interest; n=number of subjects with data available

Numbers analysed

Outcomes and estimation

Primary endpoint

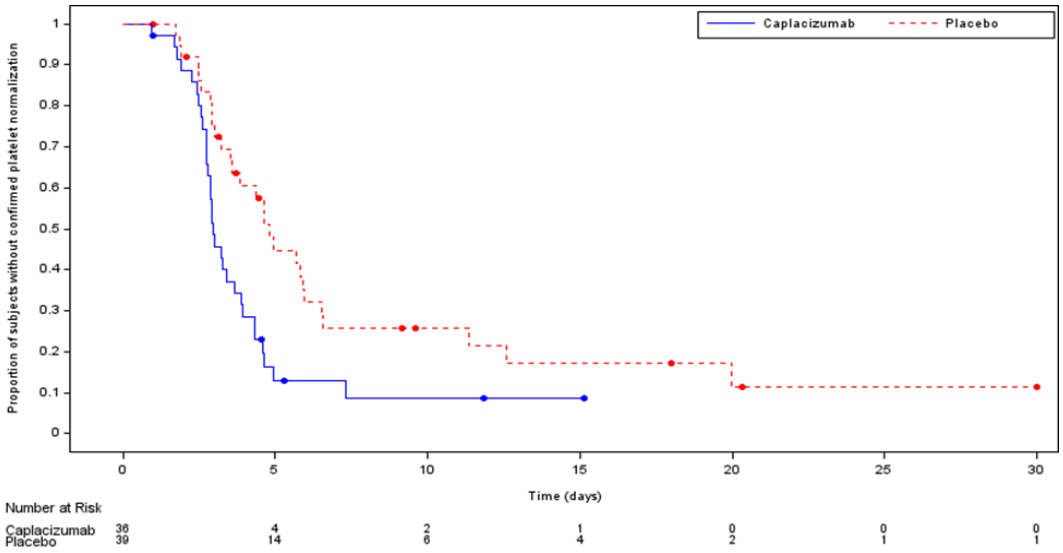
Stratified for absence/presence of one PE session prior to randomization, conducted on the ITT population. An observation was censored if it did not meet the defined time interval of 30 days after first administration of study drug, due to any cause of loss to follow-up (including death), or endpoint not being reached.

The number and proportion of subjects in each treatment group with PE tapering were similar in the caplacizumab

treatment group compared with the placebo treatment group (11 [30.6%] subjects versus 11 [28.2%] subjects).

Only 2 subjects in the experimental arm and 4 subjects in the control arm all patients had received PE prior to randomization.

Figure 4: Time to confirmed platelet response curves (ITT population)



Censored observations are represented as dots. Any subject still at risk at 30 days was censored at 30 days.

Analysis of time to confirmed platelet response (ITT population)

		ALX-0081 N=36	Placebo N=39
Overall	Censored Subjects at 30 days, n (%) [1] [2]	5 (13.9)	11 (28.2)
	Subjects with Confirmed Platelet Response, n (%) [1] [2]	31 (86.1)	28 (71.8)
One PE Session Prior to Randomisation: Yes	Censored Subjects at 30 days, n (%) [1] [2]	0	0
	Subjects with Confirmed Platelet Response, n (%) [1] [2]	2 (5.6)	4 (10.3)
	Median (95% CI)	2.44 (1.92, 2.97)	4.31 (2.91, 5.68)
	25th Percentile (95% CI)	1.92 (1.92, 2.97)	3.37 (2.91, 4.79)
	75th Percentile (95% CI)	2.97 (1.92, 2.97)	5.23 (2.91, 5.68)
One PE Session Prior to Randomisation: No	Censored Subjects at 30 days, n (%) [1] [2]	5 (13.9)	11 (28.2)
	Subjects with Confirmed Platelet Response, n (%) [1] [2]	29 (80.6)	24 (61.5)
	Median (95% CI)	3.00 (2.74, 3.88)	4.92 (3.21, 6.59)
	25th Percentile (95% CI)	2.72 (1.76, 2.85)	3.01 (1.92, 4.36)
	75th Percentile (95% CI)	4.31 (3.41, 7.31)	11.37 (5.89, DNE)
Hazard Rate Ratio (95% CI)	ALX-0081 vs. Placebo [3]	2.197 (1.278, 3.778)	
Stratified Log-Rank Test:	p-value [4]	0.005	

Notes:

[1] Response was defined by a recovery of platelets ≥ 150,000/μL. This response had to be confirmed at 48 hours after the initial reporting of platelet recovery ≥ 150,000/μL by a *de novo* measure of platelets ≥ 150,000/μL and lactate dehydrogenase ≤ 2 x the ULN.
Time to Response = [Date and time (hrs, min) of platelets ≥ 150,000/μL] – [Date and time (hrs, min) of first study drug administration]

[2] The denominator is the number of subjects in each treatment group.

[3] Hazard ratio is on a stratified Cox proportional hazards regression model with One PE Session Prior to Randomisation (Yes/No) as a covariate.

[4] p-value from stratified log-rank test is based on an analysis stratified for presence/absence of one PE session prior to randomisation.

		Caplacizumab N=36	**Placebo N=39**
Overall	Median time to response (95% CI) - days	2.97 (2.74, 3.65)	4.79 (3.51, 5.94)
	Subjects with Confirmed Platelet Response, n (%) [1][2]	31 (86.1)	28 (71.8)
Time to confirmed platelet response	p-value[3] (Stratified Log-Rank Test)	0.005	
Hazard Ratio	Caplacizumab vs. placebo[4] (95% CI)	2.197 (1.278, 3.778)	

Selected secondary outcomes

Secondary efficacy analyses in Study ALX-0681-2.1/10 (ITT population)

			Caplacizumab N=36	Placebo N=39
Complete remission [1]		n (%)	29 (80.6)	18 (46.2)
Exacerbations of aTTP [2]		n (%)	3 (8.3)	11 (28.2)
Relapse of aTTP [3]	1-month FU period	n (%)	8 (22.2)	0 (0.0)
	12-month FU period	n (%)	11 (30.6)	3 (7.7)
Exacerbation/Relapse of aTTP [2],[3]	1-month FU period	n (%)	10 (27.8)	11 (28.2)
	12-month FU period	n (%)	13 (36.1)	13 (33.3)

[1] Complete remission was defined as platelet count ≥ 150,000/µL and LDH ≤ 2 times the ULN at 48 hours after initial platelet response and absence of exacerbation.

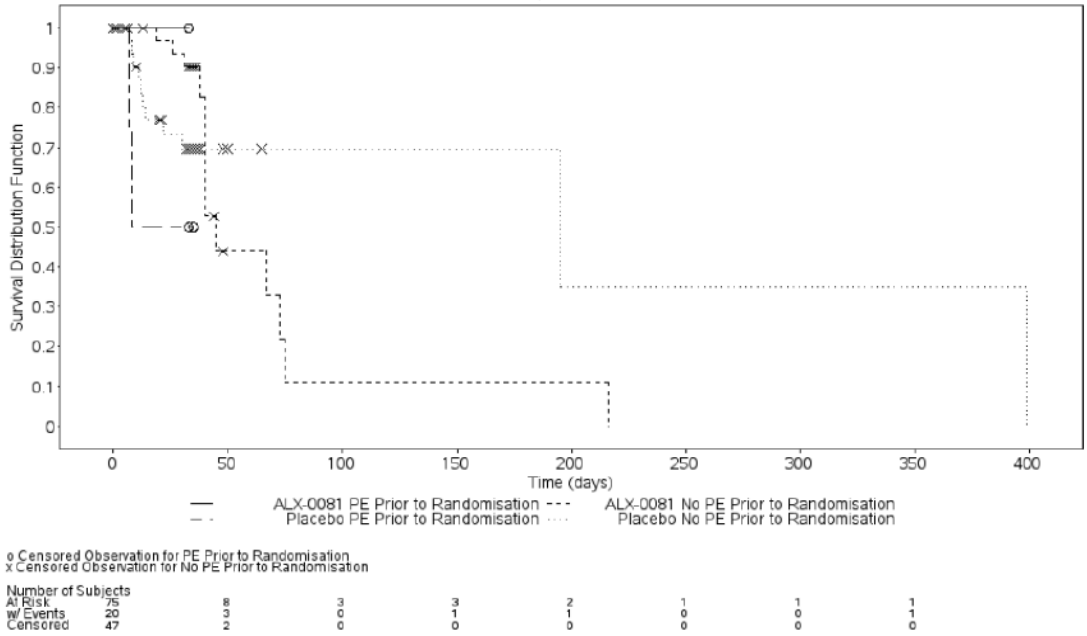
[2] An exacerbation of aTTP was defined as recurrent thrombocytopenia following a confirmed platelet response and requiring a re-initiation of daily PE treatment after ≥ 1 day but ≤ 30 days of no daily PE treatment.

[3] Relapsing of aTTP was defined as *de novo* event of aTTP that occurred later than 30 days after the last PE.

Note, 22 (61.1%) of the subjects in the caplacizumab group and 21 (53.8%) of the subjects in the placebo group were followed up to the 12-month follow-up visit

Note, the 1-month FU period was analysed post-hoc

Figure 5: Time to First Exacerbation/Relapse of Thrombotic Thrombocytopenic Purpura (ITT)



Selected post-hoc analysis

ADAMTS13 Activity in Relation to Exacerbation and Relapse – Post-Hoc Analysis (Safety population)

Severe ADAMTS13 deficiency (i.e., <10% of normal) is used to confirm the diagnosis of aTTP, and has been recognized as a potential biomarker for relapse risk during. The 10% cut-off level is increasingly considered more clinically relevant than the protocol specified cut-off of 5%. It was hypothesized that unresolved disease activity (i.e., persistent anti-ADAMTS13 antibodies), reflected by ADAMTS13 activity levels <10% during the treatment period, could account for early relapse observed in the caplacizumab group. Accordingly, a post-hoc descriptive analysis was performed to investigate this possibility. Events preceded by a continuous severe deficiency in ADAMTS13 during the treatment period were considered as a recurrence of the presenting aTTP episode ('relapse of presenting episode'). Relapses that were preceded by a normalization of ADAMTS13 to levels above 10% were considered de novo events. The relationship between low ADAMTS13 activity and exacerbation during the treatment phase was also explored. The results of this analysis are presented in Figure 4.

For subjects not experiencing a recurrence or those experiencing a relapse, the measurement of ADAMTS13 activity closest to the treatment stop date was used. For those experiencing an exacerbation, the measurement of ADAMTS13 activity data closest to or on the day of the recurrence was used in this analysis.

Subjects experiencing relapse (recurrence of aTTP during the follow-up period):

In the caplacizumab group, 11 subjects experienced a relapse during the follow-up period. The majority of them (7 subjects) relapsed within 10 days of receiving the last dose of study drug. All of these “early relapsing” subjects exhibited ADAMTS13 activity of < 10% from baseline through the last measurement on treatment, indicating ongoing disease activity (Figure 4). In contrast, all 4 subjects who experienced a first relapse more than 10 days after stopping caplacizumab had an ADAMTS13 activity ≥ 10% during and/or near the end of the treatment period, suggesting resolution of their underlying disease. Relapse in these subjects occurred much later (30-167 days after stopping caplacizumab), suggesting that it was associated with a de novo episode of aTTP, rather than a relapse of the presenting episode.

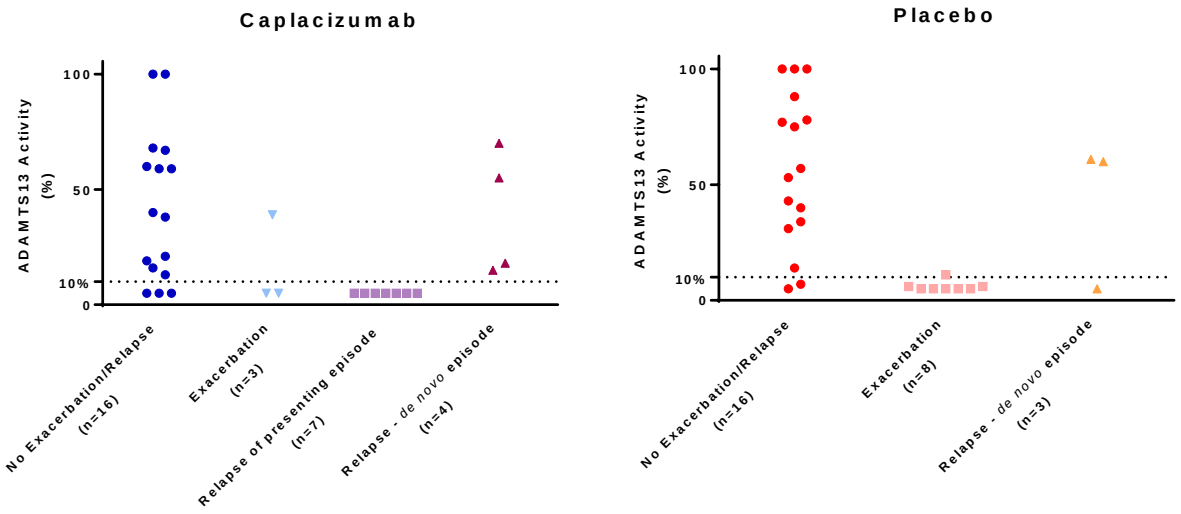
In the placebo group, 3 subjects had a relapse. All of the relapses occurred beyond the 1-month follow-up period (161-356 days after cessation of the study treatment). Two of them had ADAMTS13

activity ≥ 10% during the treatment period, suggesting a de novo episode of aTTP. One subject had a value < 10% during the study drug treatment period and at the 1-month follow-up visit. No subsequent ADAMTS13 measurements are available for this subject.

Subjects experiencing exacerbation (recurrence of aTTP during the treatment period):

In the caplacizumab group, 3 subjects had an exacerbation, and all 3 had baseline ADAMTS13 activity <10%. Of them, 2 still had ADAMTS13 activity < 10% around the time of the exacerbation (range: 0–8 days prior to exacerbation) and 1 had ADAMTS13 activity of 39% on the day of the exacerbation (Figure 4). In the placebo group, 11 subjects had an exacerbation, with baseline ADAMTS13 activity <10% in 10 of them. ADAMTS13 data around the time of exacerbation were available for 8 of these subjects (range 0-6 days prior to exacerbation); all but one had ADAMTS13 activity <10% at this time. The remaining subject had a result of 11%.

Figure 6: Evaluation of ADAMTS13 activity data in caplacizumab-treated subjects and placebo-treated subjects



Note: Subjects without ADAMTS13 activity data at the selected time points, or subjects without severe ADAMTS13 deficiency at screening and other visits up to the 1 month follow-up, or subjects who prematurely terminated from the study were excluded from this analysis.

ALX0681-C301 (Hercules) study: A Phase III double-blind, randomized, parallel group, multicenter placebo-controlled trial to study the efficacy and safety of caplacizumab in patients with acquired thrombotic thrombocytopenic purpura.

Methods

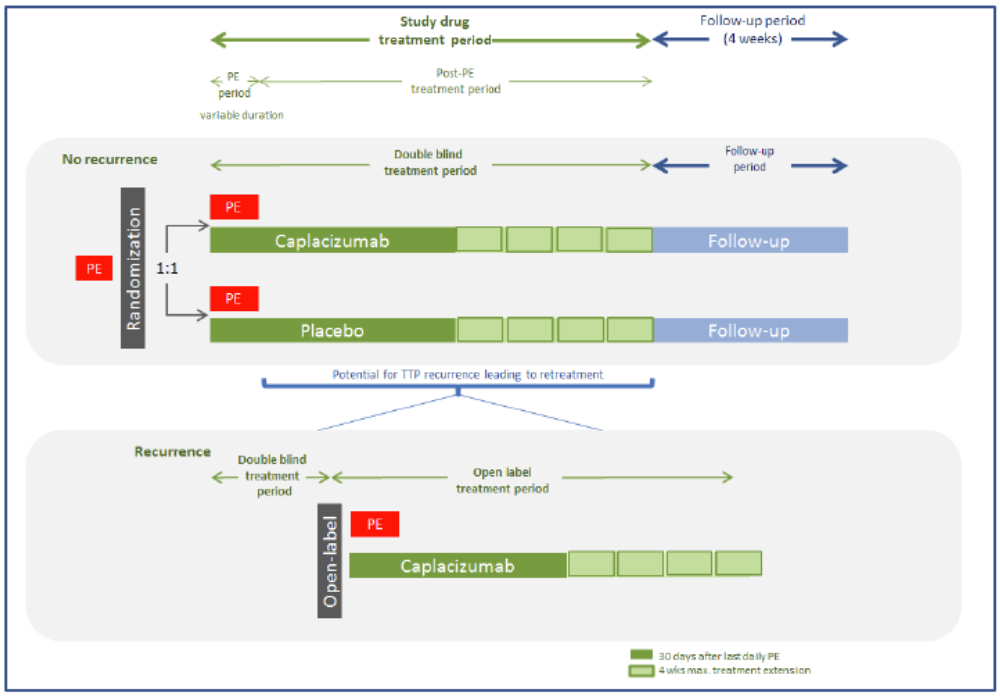
Study Design

This was a phase III, randomized, double-blind, placebo-controlled, study to evaluate the efficacy and safety of caplacizumab when administered in addition to standard of care treatment in subjects with an acute episode of acquired TTP. The study evaluated the efficacy of caplacizumab in more rapidly restoring normal platelet counts. In addition, the effect of treatment with caplacizumab on a composite endpoint of TTP-related mortality, recurrence of TTP and major thromboembolic events during study drug treatment and on endpoints assessing recurrence of TTP during the overall study period, refractory TTP and time to normalization of organ damage marker levels, were evaluated. After

confirmation of eligibility to study participation and after the start of PE treatment (the maximum time allowed between the start of first PE (i.e., PE administered prior to randomization) and the start of the first PE after randomization (i.e., the first on-study PE) was 24 hours), subjects were randomized in a ratio of 1:1 to receive caplacizumab or placebo in addition to standard of care therapy. Randomization was stratified by severity of neurological symptoms (Glasgow coma scale [GCS]).

All patients received the study drug up to 30 days after the end of daily PE. Risk-guided continuation for up to 28 additional days was possible in conjunction with optimized immunosuppression.

In the case of exacerbation or recurrence during the treatment period all patients were switched to open label caplacizumab. This complicates interpretation of study outcomes. Relapse during FU was treated according to standard of care. There was no re-initiation of study drug administration for subjects experiencing more than one exacerbation or relapse.



Study Participants

Adults with a clinical diagnosis of acquired TTP who required initiation of daily PE treatment.

<u>The main criteria for inclusion were the following:</u>

- Adult male or female ≥ 18 years of age at the time of signing the informed consent form (ICF)†
- Clinical diagnosis of acquired TTP (initial or recurrent), which included thrombocytopenia and microscopic evidence of red blood cell fragmentation (e.g., schistocytes)
- Required initiation of daily PE treatment and had received 1 PE treatment‡ prior to randomization

<u>The main criteria for exclusion were the following:</u>

- Platelet count ≥100×10^9/L

- Serum creatinine level >200 µmol/L in case platelet count was > 30×10^9/L (to exclude possible cases of atypical Hemolytic Uremic Syndrome [aHUS])
- Known other causes of thrombocytopenia including but not limited to:
    - Clinical evidence of enteric infection with E. coli 0157 or related organism
    - Atypical HUS
    - Hematopoietic stem cell, bone marrow or organ transplantation-associated thrombotic microangiopathy
    - Known or suspected sepsis
    - Diagnosis of disseminated intravascular coagulation
    - Congenital TTP (known at the time of study entry)
- Pregnancy or breast-feeding
- Clinically significant active bleeding or high risk of bleeding (excluding thrombocytopenia)
- Known chronic treatment with anticoagulant treatment that could not be stopped (interrupted) safely, including but not limited to:
    - vitamin K antagonists
    - heparin or low molecular weight heparin (LMWH)
    - non-acetyl salicylic acid non-steroidal anti-inflammatory molecules
- Malignant arterial hypertension
- Clinical condition other than that associated with TTP, with life expectancy < 6 months, such as end-stage malignancy
- Subjects who were previously enrolled in a clinical study with caplacizumab and received caplacizumab or for whom the assigned treatment arm was unknown

Treatments

The study medication was provided in a kit containing the following components:

- One glass vial containing lyophilized powder for reconstitution (containing either caplacizumab or placebo).
- One prefilled glass syringe containing solvent for reconstitution (containing water for injection [WFI]).
- One “vial adapter” device to facilitate transfer of the solvent for reconstitution and subsequent recovery of the reconstituted drug.
- One safety needle for s.c. use (please note that a needle for the first i.v. bolus injection was not included in the kit).
- Two alcohol pads.

Objectives

Primary Objective:

- To evaluate efficacy of caplacizumab in more rapidly restoring normal platelet counts as measure of prevention of further microvascular thrombosis

Key Secondary Objectives, hierarchically ordered:

- to evaluate the effect of study drug on a composite endpoint consisting of thrombotic thrombocytopenic purpura (TTP)-related mortality, recurrence of TTP and major thromboembolic events during study drug treatment

- to evaluate the effect of study drug on prevention of recurrence of TTP over the entire study period

- to evaluate the effect of study drug on refractoriness to treatment

- to evaluate the effect of study drug on biomarkers of organ damage: lactate dehydrogenase (LDH), cardiac troponin I (cTnI), and serum creatinine

Other Secondary Objectives:

- to evaluate the effect of study drug on plasma exchange (PE) parameters (days of PE and volume), days in intensive care unit (ICU), days in hospital

- adverse events (AEs)

- pharmacodynamic (PD) markers: von Willebrand factor antigen (vWF:Ag), coagulation factor VIII clotting activity (FVIII:C), ristocetin cofactor activity (RICO)

- pharmacokinetic (PK) parameters

- immunogenicity (anti-drug antibodies [ADA])

Outcomes/endpoints

Primary: Time to platelet count response defined as initial platelet count ≥ 150×10^9/L with subsequent stop of daily PE within 5 days.

Key secondary endpoints

The key secondary endpoints are hierarchically ordered as listed below:

- Proportion of subjects with TTP-related death, a recurrence of TTP, or at least one treatment-emergent major thromboembolic event (e.g., myocardial infarction, cerebrovascular accident, pulmonary embolism or deep venous thrombosis [DVT]) during the study drug treatment period (including extensions).
- Proportion of subjects with a recurrence of TTP in the Overall Study Period (including 4-week FU period).
- Proportion of subjects with refractory TTP, defined as absence of platelet count doubling after 4 days of standard treatment, and LDH > upper limit of normal (ULN).
- Time to normalization of all 3 of the following organ damage marker levels:
    - Time to LDH ≤ 1 x ULN, and cTnI ≤ 1 x ULN, and serum creatinine ≤ 1 x ULN

Other secondary endpoints

- Proportion of subjects with recurrences of TTP as well as the number of such events during study drug treatment (including extensions) and after end of study drug treatment
- PE parameters: number of days and total plasma volume (absolute and normalized) in 2 time periods: initial daily PE period and full study drug treatment period
- Number of days in ICU and in hospital in 4 time periods: initial daily PE period, full study drug treatment period, in the FU period (of 4 weeks after stop of study treatment) and Overall Study Period

Sample size

The planned sample size was approximately 132 adult subjects in 2 arms, randomized in a 1:1 ratio and stratified by severity of neurological involvement prior to randomization (GCS score ≤ 12 versus GCS score = 13 - 15). A total of 145 subjects (72 subjects to caplacizumab and 73 subjects to placebo) were enrolled (i.e., randomized) in the study. 71 subjects in the caplacizumab group and 73 in the placebo group received at least one dose of study drug and were included in the Safety Population and in the mITT population. One subject randomized to caplacizumab withdrew consent prior to first dosing.

Randomisation

Subjects were randomized to one of the 2 arms in a 1:1 ratio. Randomization was stratified by severity of neurological involvement (GCS ≤ 12 vs. GCS = 13-15). Stratification was foreseen to ensure balanced treatment arms for the secondary endpoints related to neurological involvement and not for the primary endpoint, for which the stratification parameter is not known to be relevant.

Blinding (masking)

In order to protect the integrity of the data, treatment assignment was kept blinded for investigational sites, subjects, site monitors, and other members of the study team, until the final database lock (when the last subject had completed the final FU visit and all data was considered clean).

Note that all subjects experiencing a recurrence during the study drug treatment period subsequently received caplacizumab in an open-label design, irrespective of initial treatment allocation and without breaking the blind for the initial treatment allocation.

Statistical methods

Intent-to-treat population: All subjects who were randomized.

The ITT population was used for selected general outputs (e.g., disposition) and for the main efficacy analysis.

Modified intent-to-treat population: All randomized subjects who received at least 1 administration of study drug, as randomized.

The modified ITT (mITT) population was used for selected (sensitivity) analysis of efficacy.

Per-protocol (PP) population: The PP population is a subpopulation of the ITT population, excluding those subjects who had a major protocol deviation.

Safety Population: All subjects who received at least 1 administration of study drug, as treated.

The Safety Population was used for analysis of safety.

Analyses were handled differently before and after switch to open-label caplacizumab after recurrence, as specified below.

Before switch to open-label caplacizumab the treatment group as assigned by the randomization was used (i.e., as-randomized analysis) for efficacy. For safety, PK, PD, disease-related markers and immunogenicity analyses the treatment that was actually used by the subject was applied (i.e., as-treated analysis).

Differences between as-treated and as-randomized were flagged in the listing on subject allocation.

After switch to open-label caplacizumab an all-treated analysis was conducted in a separate pooled open-label caplacizumab treatment group, unless specified otherwise. For some specific analyses, e.g., for immunogenicity analyses, this pooling was not done. As a consequence, if open-label period is represented according to the randomized treatment, the actual treatment (Caplacizumab) can differ from represented treatment group (Placebo or Caplacizumab).

Results

Participant flow

Figure 7: Subject disposition in Study ALX0681-C301

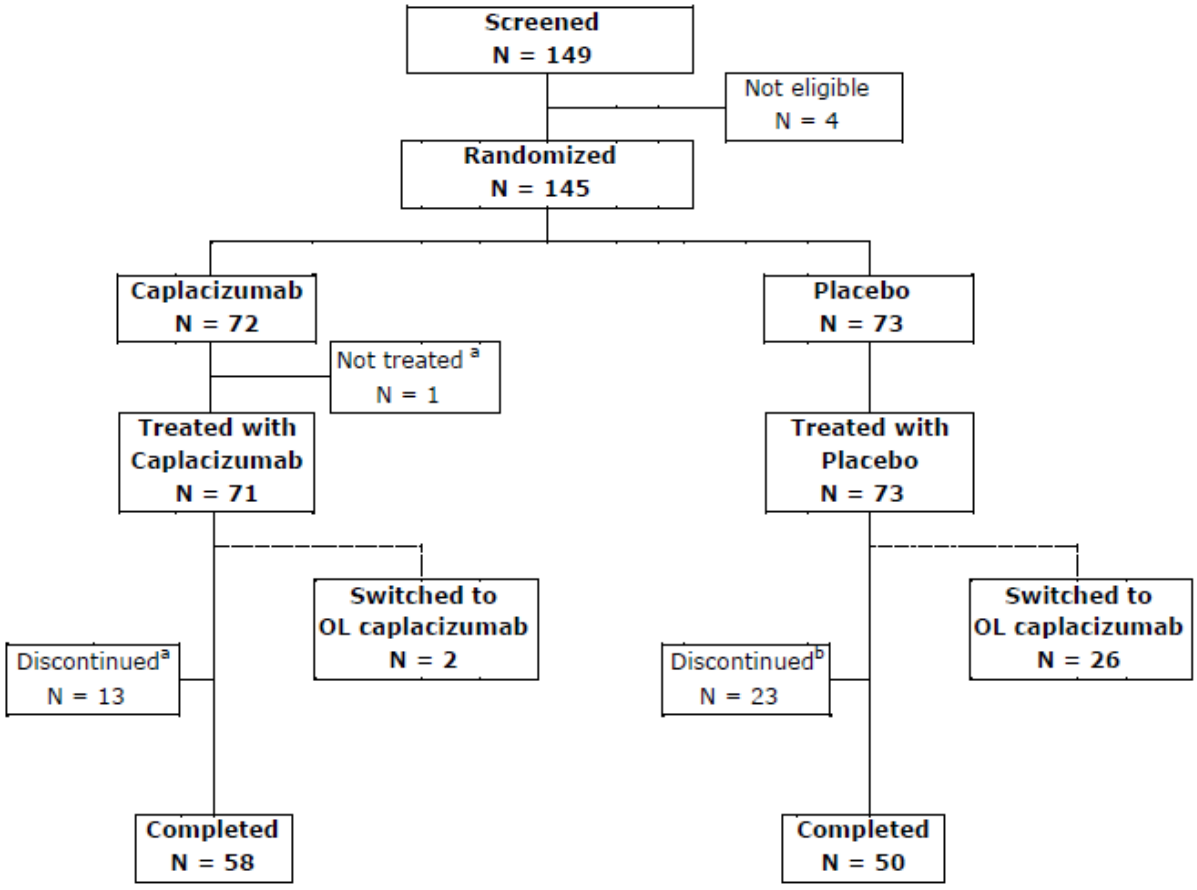


Figure 7: Subject disposition in Study ALX0681-C301

[a]: Of the 14 subjects who discontinued in the caplacizumab group, 6 subjects performed their final FU visit.
[b]: Of the 23 subjects who discontinued in the placebo group, 10 subjects performed their final FU visit.

Treatment extension beyond 30 days after end of daily PE was observed for 20 subjects in the caplacizumab arm and 5 in the placebo group; here, the considerably higher number of subjects switching to caplacizumab OL in the placebo arm must be considered (26 vs 2 in the caplacizumab arm).

In the caplacizumab arm, 9 subjects received the maximum duration of treatment, 30 + 28 days, 5 subjects had 3 weeks of treatment extensions, 4 subjects had 2 weeks of treatment extensions and 2 subjects had 1 week of treatment extension.

Table 8: Summary table of treatment extensions and study terminations (ITT Population)

Table 7: Summary table of treatment extensions and study termination (ITT Population)

Population, n (%)	Caplacizumab (N = 72)	Placebo (N = 73)	All subjects (N = 145)
Completed the DB Week 5 visit (i.e., end of 30-day post-daily PE Period)	60 (83.3)	33 (45.2)	93 (64.1)
Had at least 1 week of DB treatment extensions [a]	20 (27.8)	5 (6.8)	25 (17.2)
1 week of DB treatment extensions	2 (2.8)	1 (1.4)	3 (2.1)
2 weeks of DB treatment extensions	4 (5.6)	0	4 (2.8)
3 weeks of DB treatment extensions	5 (6.9)	1 (1.4)	6 (4.1)
4 weeks of DB treatment extensions	9 (12.5)	3 (4.1)	12 (8.3)
Completed the Overall Study Period	58 (80.6)	50 (68.5)	108 (74.5)
Discontinued	14 (19.4)	23 (31.5)	37 (25.5)
Primary reason for discontinuation:			
Adverse event	6 (8.3)	5 (6.8)	11 (7.6)
Subject lost to follow-up	0	1 (1.4)	1 (0.7)
Non-compliance with study drug	0	1 (1.4)	1 (0.7)
Subject withdrew consent	4 (5.6)	5 (6.8)	9 (6.2)
Legal representative withdrew consent	0	1 (1.4)	1 (0.7)
Death	1 (1.4)	3 (4.1)	4 (2.8)
Physician's decision	2 (2.8)	4 (5.5)	6 (4.1)
Other	1 (1.4)	3 (4.1)	4 (2.8)

Abbreviations: DB = Double-blind; N = number of subjects within the population of interest (by treatment group); n = number of subjects with non-missing observations

[a] 3 subjects, i.e., 2 subjects in the caplacizumab group (Subjects 3010099 and 3010107) and 1 subject in the placebo group (Subject 3010091) started treatment extensions but discontinued the study prior to reaching 1 week of treatment extensions (i.e., having a Week 6 visit).

Any major protocol deviation was reported for 45% of subjects in the caplacizumab arm and 43% in the placebo arm with "treatment non-compliance" being the most prevalent reason. However, the protocol deviations are not deemed likely to favour the experimental arm or to challenge the overall results of the study.

Recruitment

Of the 92 investigational sites that were initiated, 55 sites in 15 countries enrolled (i.e. randomized) subjects: Australia (3 centres), Austria (1 centre), Belgium (4 centres), Canada (4 centres), Czech Republic (2 centres), France (6 centres), Hungary (2 centres), Israel (4 centres), Italy (5 centres), The Netherlands (1 centre), Spain (6 centres), Switzerland (1 centre), Turkey (3 centres), United Kingdom (3 centres) and the United States of America (10 centres).

Consent was obtained from the first subject on 19 Nov 2015, the last subject completed the final visit on 16 Aug 2017.

Overall, 108 subjects (74.5%) completed the study (i.e., completed treatment and had their final follow-up visit) and 37 subjects (25.5%) discontinued from the study.

Conduct of the study

Overall, 64 subjects (44.1%) had a major protocol deviation; 31 subjects (43.1%) in the caplacizumab group and 33 subjects (45.2%) in the placebo group. Overall, the most commonly reported category of major protocol deviations was “treatment non-compliance”, reported in 36 subjects (24.8%) (caplacizumab group: 15 subjects [20.8%]; placebo group: 21 subjects [28.8%]), followed by “selection criteria not met” reported in 21 subjects (14.5%) (caplacizumab group: 11 subjects [15.3%]; placebo group: 10 subjects [13.7%]).

There were two protocol amendments relating to:

- A change in exclusion criteria: to exclude subjects who were previously enrolled in a clinical study with caplacizumab and received caplacizumab or for whom the assigned treatment arm is unknown and removal of the planned interim analysis
- A change in planned sample size (increased from 92 to 132 to account for a change in the assumed treatment difference for the primary endpoint in the sample size calculation)
- The inclusion of subjects aged 2 to 18 years

Baseline data

Table 9: Demographic data: Descriptive statistics (ITT population)

	Caplacizumab (N = 72)	Placebo (N = 73)	All Subjects (N = 145)
Baseline Platelet count (10^9/L) [a]			
N	70	72	142[b]
Mean (SD)	32.0 (27.19)	39.1 (29.13)	35.6 (28.32)
Median (Min; Max)	24.0 (3; 119)	25.5 (9; 133)	24.0 (3; 133)
Previous TTP episodes, n (%)	72	73	145
Initial	48 (66.7)	34 (46.6)	82 (56.6)
Recurrent	24 (33.3)	39 (53.4)	63 (43.4)
Number of previous TTP episodes, n (%)	72	73	145
0	48 (66.7)	34 (46.6)	82 (56.6)
1	8 (11.1)	21 (28.8)	29 (20.0)
2	9 (12.5)	7 (9.6)	16 (11.0)
>2	7 (9.7)	11 (15.1)	18 (12.4)
Severity of disease, n (%)	72	73	145
Very severe	30 (41.7)	25 (34.2)	55 (37.9)
Less severe	42 (58.3)	48 (65.8)	90 (62.1)
ADAMTS13 activity, n (%)	71	72	143
< 10%	58 (81.7)	65 (90.3)	123 (86.0)
≥ 10%	13 (18.3)	7 (9.7)	20 (14.0)
missing	1	1	2
cTnI, n (%)	66	66	132
≤ ULN	30 (45.5)	31 (47.0)	61 (46.2)
> ULN	36 (54.5)	35 (53.0)	71 (53.8)
Missing	6	7	13
cTnI (µg/L)			
n	66	66	132
Mean (SD)	3.4658 (13.59726)	0.6330 (1.57362)	2.0494 (9.74614)
Median (Min; Max)	0.0880 (0.010; 75.959)	0.0690 (0.010; 7.282)	0.0785 (0.010; 75.959)
Lactate Dehydrogenase, n (%)	66	66	132
≤ ULN	7 (10.6)	10 (15.2)	17 (12.9)
> ULN	59 (89.4)	56 (84.8)	115 (87.1)
Missing	6	7	13
Lactate Dehydrogenase (U/L)			
n	66	66	132
Mean (SD)	612.9 (445.45)	517.4 (457.38)	565.2 (452.28)
Median (Min; Max)	449.5 (120; 2525)	403.0 (151; 3343)	422.0 (120; 3343)
Serum creatinine, n (%)	66	66	132
≤ ULN	53 (80.3)	49 (74.2)	102 (77.3)
> ULN	13 (19.7)	17 (25.8)	30 (22.7)
Missing	6	7	13
Serum creatinine (µmol/L)			
n	66	66	132
Mean (SD)	100.015 (99.9434)	102.030 (70.6695)	101.023 (86.2280)
Median (Min; Max)	77.000 (35.00; 717.00)	82.500 (52.00; 482.00)	80.000 (35.00; 717.00)
GCS score, n (%)	71	72	143
≤ 12	6 (8.5)	5 (6.9)	11 (7.7)
13-15	65 (91.5)	67 (93.1)	132 (92.3)
Missing	1	1	2
SMMSE total score			
n	62	61	123
Mean (SD)	24.8 (8.88)	25.3 (6.32)	25.0 (7.69)
Median (Min; Max)	28.0 (0; 30)	27.0 (0; 30)	28.0 (0; 30)

	Caplacizumab (N = 72)	Placebo (N = 73)	All Subjects (N = 145)
RICO activity, %			
n	66	64	130
Mean (SD)	123.46 (53.401)	135.04 (68.443)	129.16 (61.306)
Median (Min; Max)	123.10 (11.0; 300.0)	126.75 (11.8; 300.0)	124.45 (11.0; 300.0)
vWF:Ag concentration, %			
n	65	64	129
Mean (SD)	159.49 (52.381)	174.52 (79.754)	166.95 (67.524)
Median (Min; Max)	158.60 (32.0; 312.5)	164.70 (22.5; 559.2)	162.30 (22.5; 559.2)
FVIII:C activity, %			
n	60	62	122
Mean (SD)	179.43 (61.709)	168.35 (74.583)	173.80 (68.499)
Median (Min; Max)	177.30 (32.0; 335.9)	166.85 (45.3; 409.2)	173.00 (32.0; 409.2)
Baseline C5a (mg/L), %			
n	66	62	128
Mean (SD)	0.014 (0.0110)	0.017 (0.0375)	0.016 (0.0272)
Median (Min; Max)	0.011 (0.00; 0.07)	0.013 (0.00; 0.30)	0.012 (0.00; 0.30)
Baseline C5b-9 (mg/L), %			
n	64	62	126
Mean (SD)	0.38 (0.276)	0.26 (0.175)	0.32 (0.237)
Median (Min; Max)	0.27 (0.1; 1.3)	0.22 (0.1; 1.0)	0.25 (0.1; 1.3)

Abbreviations: ADAMTS13 = A disintegrin-like and metalloprotease with thrombospondin repeats 13; cTnI = cardiac troponin I; FVIII = coagulation factor VIII; GCS = Glasgow Coma Scale; Min = minimum; Max = Maximum; N = number of subjects within the population of interest (by treatment group); n = number of subjects with non-missing observations; RICO = ristocetin cofactor activity; SD = standard deviation; SMMSE = Standardized mini mental state examination; TTP = thrombotic thrombocytopenic purpura; vWF:Ag = von Willebrand factor antigen

Note: The denominator for the percentage calculations is the total number of subjects per treatment group in the ITT population, excluding missing values.

[a] Of note, screening platelet counts were according to the study's entry criteria (i.e., $<100 \times 10^9$/L), except for 1 subject (Subject 3010122; placebo group) who entered the study with a platelet count of 100×10^9/L, which was reported as a major protocol deviation.

[b] For 2 of the 3 subjects with missing baseline platelet count, the first sampling for platelet count occurred after the subjects received the first dose of study drug and for the third subject, a screening value but no baseline value was available.

Generally, baseline data is not likely to favour the experimental arm. A higher fraction of subjects with a recurrent episode is noted in the placebo arm. For 7 subjects with baseline ADAMTS13 activity of ≥10% (4 subjects in the caplacizumab group and 3 subjects in the placebo group), the diagnosis of aTTP could not be confirmed based on prior medical history of TTP or other available information including subsequent measurements of ADAMTS13 activity.

Immunosuppressive medications

During the overall study period, slightly more patients in the placebo arm received rituximab (49% vs 40%) while other immunosuppressive medication, mainly MMF, was more commonly delivered in the caplacizumab arm (14% vs 6%).

During the DB daily PE period 30% of subjects in the placebo arm vs 17% in the caplacizumab arm received rituximab.

During the DB post daily PE period roughly equal fractions in the study arms received rituximab, 35-37%.

For context, during the DB treatment period, the median (min; max) duration of study drug treatment was 35 (1; 65) days for the DB caplacizumab group and 23 (2; 66) days for the DB placebo group.

During the OL treatment period >40% of subjects received rituximab.

During the FU period immunosuppressive medications were taken by a considerably larger fraction of subjects in the caplacizumab arm, 75% vs 52% in the placebo group, while rituximab was used by

18% and 25%, respectively. Given the option of OL caplacizumab, considerably more used in the placebo arm, these figures are very difficult to interpret.

Numbers analysed

Outcomes and estimation

Table 10: Summary table by analysis population

Data Sets Analyzed

Population, N (%)	Caplacizumab (N = 72)	Placebo (N = 73)	All subjects (N = 145)
Intent-to-treat	72 (100)	73 (100)	145 (100)
Modified Intent-to-treat	71 (98.6)	73 (100)	144 (99.3)
Per protocol	41 (56.9)	40 (54.8)	81 (55.9)
Safety	71 (98.6)	73 (100)	144 (99.3)
Open-label caplacizumab	2 (2.8)	26 (35.6)	28 (19.3)

N = number of subjects within the population of interest (by treatment group)

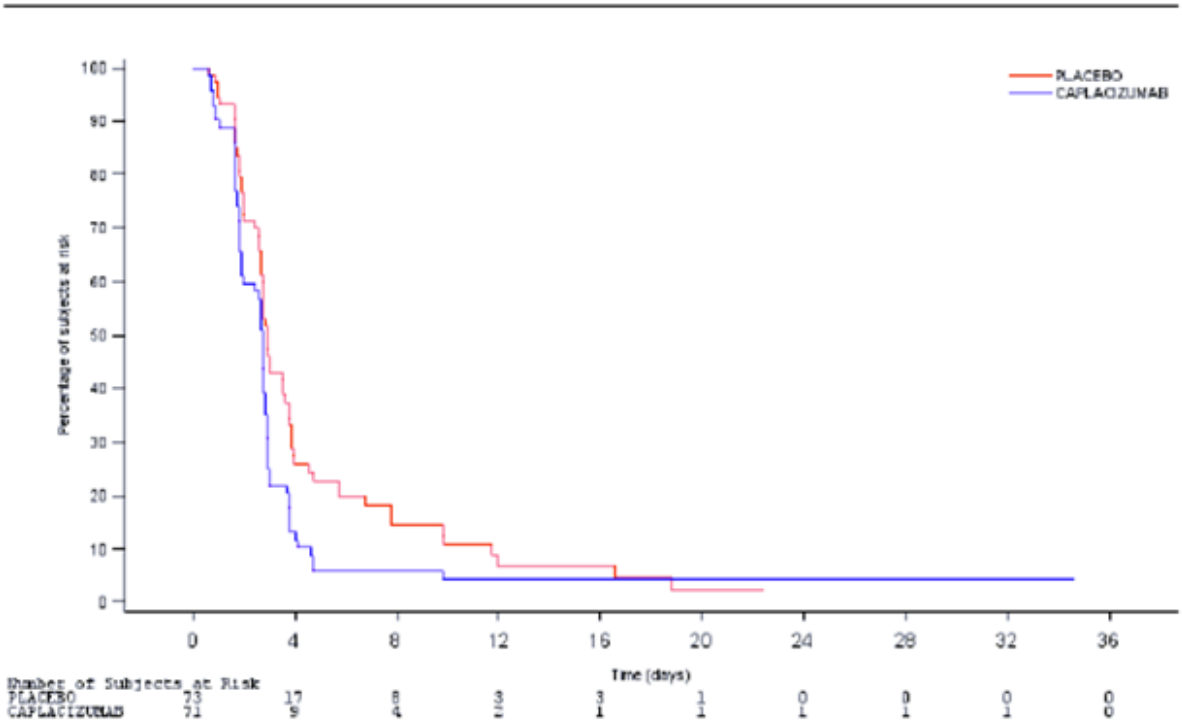
Primary Endpoint: Time to Confirmed Platelet Response

POPULATION: INTENT-TO-TREAT
PART 1: OVERALL

SUMMARY STATISTIC	PLACEBO (N=73)	CAPLACIZUMAB (N=72)
TIME TO PLATELET COUNT RESPONSE		
Median (95% CI)	2.88 (2.68; 3.56)	2.69 (1.89; 2.83)
25% Percentile (95% CI)	1.94 (1.70; 2.64)	1.75 (1.65; 1.87)
75% Percentile (95% CI)	4.50 (3.78; 7.79)	2.95 (2.85; 3.81)
Number assessed	73	71
Number censored	7	5
Number events	66	66
Stratified Logrank test (p-value)		0.0099
Hazard Ratio (95% CI) [1]		1.55 (1.095; 2.195)

[1] COX PROPORTIONAL HAZARDS MODEL WITH TREATMENT GROUP AND GCS CATEGORY AS INDEPENDENT VARIABLES, A HAZARD RATIO > 1 INDICATES AN ADVANTAGE OF CAPLACIZUMAB.
TIME TO EVENT IS CALCULATED FROM FIRST STUDY DRUG ADMINISTRATION
STRATIFICATION FACTOR IS GLASGOW COMA SCALE (GCS)
IF A TIME-TO-EVENT PERCENTILE OR CORRESPONDING C.I. DOES NOT EXIST (DUE TO TOO SMALL NUMBER OF EVENTS), IT IS REPLACED BY A DOT.

Figure 8: Time to confirmed platelet response curves (ITT population)



Source: Figure 14.2.1.1.1

Subgroup analyses

The HR for time to platelet count response was

- 1.59 (1.017-2.473) in subjects with less severe disease and 1.69 (0.936-3.038) in subjects with very severe disease;
- 1.67 (1.025-2.722) in subjects with initial episode of TTP and 1.64 (0.951-2.818) in subjects with recurrent episode, and;
- 1.70 (1.162-2.489) in subjects with ADAMTS13 <10% at baseline and 1.52 (0.469-4.919) in subjects with ADAMTS13 ≥10% at baseline.

As pointed out by the Applicant the results on patients with ADAMTS13 activity ≥10% should be interpreted with caution as a diagnosis of aTTP could not be confirmed in 7 of them.

Based on 66 events in each arm time to confirmed platelet response was statistically significantly shorter in the caplacizumab arm, p=0.0099, with a HR of 1.55 (1.095; 2.195). In terms of medians, the absolute outcomes as well as the difference between study arms were much smaller than anticipated in the sample size calculations, only 0.19 days (2.69 vs 2.88). However, looking at the KM graph the median is not informative. Rather, the effect of caplacizumab is noted primarily in “late” responders, >3-4 days. Opposed to the phase II study, patients were required to have received one PE treatment before randomization, which likely contributed to the shorter times to platelet response observed in the phase III study. The result of the primary analysis is supported by various sensitivity analyses. The results of the provided subgroup analyses show generally consistent treatment effects.

KEY SECONDARY ENDPOINTS

- **TTP-Related Death, Recurrence of TTP, and Major Thromboembolic Events During the Study Drug Treatment Period**

Table 11:

Table 13: Percentage of subjects with TTP-related death, a recurrence of TTP, or at least one treatment-emergent major thromboembolic event during the Overall Study Drug Treatment Period (ITT population)

	Double-Blind Caplacizumab (N = 71)	Double-Blind Placebo (N = 73)
Number of subjects with, n (%)		
TTP-related death	0	3 (4.1)
Recurrence of TTP (exacerbation)	3 (4.2)	28 (38.4)
At least one treatment-emergent major thromboembolic event	6 (8.5)	6 (8.2)
Total (at least one of the above mentioned events)	**9 (12.7)**	**36 (49.3)**

N = number of subjects within the population of interest (by treatment group); n = number of subjects with events; TTP = thrombotic thrombocytopenic purpura

The outcome of this analysis, p<0.0001, is principally driven by the considerably larger number of exacerbations in the placebo arm, 28 vs 3. Regarding at least one treatment-emergent major thromboembolic event no difference between study arms was noted. In the caplacizumab group one patient died from TTP after the study drug treatment period.

- **Proportion of subjects with a recurrence of TTP in the overall study period**

The alpha-protected analysis regards proportion of subjects with a recurrence of TTP in the overall study period: 38% of subjects in the placebo arm and 13% in the caplacizumab arm, p=0.0004. In the placebo arm, all 28 recurrences (exacerbations) occurred during the DB treatment period while in the caplacizumab arm, 3 patients had an exacerbation during the DB treatment period and, notably, 6 subjects relapsed during FU. Again, interpretation of data after the DB period is very difficult due to the option of OL caplacizumab. Of note, all patients relapsing during FU had an ADAMTS13 activity level of <10% when study drug treatment was ended.

- **Proportion of subjects with refractory TTP**

The alpha-protected analysis regards proportion of subjects with refractory TTP defined as absence of platelet count doubling after 4 days of standard treatment and LDH>ULN: 3 subjects in the placebo arm vs 0 in the caplacizumab arm, p=0.0572.

Using the International Consensus Definition of refractoriness, 5 subjects in the placebo arm vs none in the caplacizumab fulfilled the criteria, descriptive p=0.0178.

- **Time to normalization of all 3 organ damage markers (i.e., LDH, cTnI and serum creatinine) for the ITT population**

Median time to normalization of LDH, cTnI and serum creatinine was numerically shorter in the caplacizumab arm, 2.86 days vs 3.36 days in the placebo arm. Due to the hierarchical testing procedure this analysis should be considered descriptive only.

OTHER SECONDARY EFFICACY ENDPOINTS

- **Plasma Exchange**

Table 12: Summary of number of days of PE and total volume of PE during the Overall Study Drug Treatment Period (ITT population)

	Caplacizumab N=72	Placebo N=73
Overall Study Drug Treatment Period (includes the OL Study Drug Treatment Period) [a]		
n	71	73
Mean (SE)	5.8 (0.51)	9.4 (0.81)
Median (Min; Max)	5.0 (1; 35)	7.0 (3; 46)
Overall Study Drug Treatment Period (includes the OL Study Drug Treatment Period) [a]		
n	71	73
Mean (SE)	21.33 (1.619)	35.93 (4.169)
Median (Min; Max)	18.06 (5.3; 102.2)	26.94 (4.0; 254.0)

Median number of days of PE during the overall study drug treatment period was 5 in the caplacizumab arm and 7 in the placebo arm, and absolute total volume of PE was 18 and 27 Liters, respectively.

As a reminder, "the 'overall treatment period' corresponds with the treatment analysis phase and covers both the double-blind (DB) treatment analysis period and the OL treatment analysis period. Note that only subjects who experienced a relapse or exacerbation had an OL treatment analysis period. For these subjects, the overall treatment period starts at start of DB treatment period and ends at end of OL treatment period. For subjects with no switch to open-label caplacizumab, the overall treatment period starts at start of DB treatment period and ends at end of DB treatment period".

- **Mortality rate**

In the placebo arm, 3 patients died during the daily PE period, all considered TTP-related. In the caplacizumab arm, one patient died during FU, also considered TTP-related.

- **Treatment-emergent major thromboembolic events during the Overall Study Drug Treatment Period (ITT population)**

Treatment-emergent major thromboembolic events during the overall study drug period were reported in 6 patients in each study arm, mainly DVT and cerebrovascular accident.

- **Number of days in ICU and hospital during the Overall Study Drug Treatment Period (ITT Population)**

The median number of days in the ICU and in hospital during the overall study drug treatment period was both numerically lower in the caplacizumab arm, 3 vs 5 and 9 vs 12, respectively.

ADAMTS13 activity

At screening, mean (SE) ADAMTS13 activity was 8.37% (2.34) in the caplacizumab group and 7.81% (2.40) in the placebo group.

The confounding of PE on ADAMTS13 activity levels and the high number of patients that switched to OL treatment in the placebo group render group-wise comparisons of ADAMTS13 activity levels over time largely non-informative.

However, looking at ADAMTS13 activity at the week after end of daily PE in platelet responders (90% in the caplacizumab group, 88% in the placebo group) the fraction of subjects reaching activity >10% was only slightly higher in the placebo group, 45% vs 38% in the caplacizumab group. This information is important as days of daily plasma exchange and plasma exchange volumes were lower in the caplacizumab group.

Population	Caplacizumab (N = 72)	Placebo (N = 73)	All subjects (N = 145)
Completed the daily PE period (i.e., had a platelet count response and end of daily PE within 5 days)	65 (90.3)	64 (87.7)	129 (89.0)
With ADAMTS13 > 10%	25 (38.5)	29 (45.3)	54 (41.9)
• Exacerbation	0	2	2
• No exacerbation	25	27	52
With ADAMTS13 ≤ 10%	**40 (61.5)**	**35 (54.7)**	**75 (58.1)**
• Exacerbation	3	26	29
• No exacerbation	37	9	46

Of the 3 patients on the caplacizumab arm with an exacerbation one subject was non-compliant with therapy while having low ADAMTS13.

The results of also the HERCULES study emphasises the need for monitoring and further immunosuppressive treatment of disease activity (e.g. ADAMTS13 levels).

Immunogenicity results

Table 13: Incidences over the different treatment groups, analysis periods and bioanalytical methods (Safety Population)

Antibody type; n (%)	Double Blind Period		Open Label Period		Overall Study Period
	Placebo (N=73)	Caplacizumab (N=71)	OL after Placebo (N=26)	OL after Caplacizumab (N=2)	All treated with Caplacizumab (N=97)
Pre-Ab positive	46 (63.0)	43 (60.6)	12 (46.2)	2 (100)	55 (56.7)
Drug induced TE ADA positive	1 (1.4)	2 (2.8)	1 (3.8)	0	3 (3.1)
TE NAb positive (with alternative NAb assay)	1 (1.4)	2 (2.8)	2 (7.7)	0	4 (4.1)
TE NAb positive (with functional NAb assay)	0	1 (1.4)	1 (3.8)	0	2 (2.1)

Abbreviations: Ab=antibody; ADA=anti-drug antibodies; NAb=neutralizing antibody; OL=open-label; TE=treatment-emergent

TE ADA responses and TE Nab detection were noted at higher frequencies in the caplacizumab group as compared to placebo, but absolute numbers were low. No influence of pre-Ab or TE ADA on time to platelet count response was found. No TE ADA were detected in any of the 3 subjects with exacerbation in the caplacizumab arm.

Ancillary analyses

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as

well as the benefit risk assessment (see later sections).

Table 14: Summary of efficacy for trial ALX-0681-2.1/10

Title: A Phase II, single-blind, randomised, placebo-controlled trial to study the efficacy and safety of anti-von Willebrand factor Nanobody administered as adjunctive treatment to patients with acquired thrombotic thrombocytopenic purpura.			
Study identifier	ALX-0681-2.1/10 (Titan)		
Design	Single-blind, randomized vs. placebo		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Treatment 30 (+) days after last PE N/A FU at 1 and 12 months
Hypothesis	Superiority		
Treatments groups	Caplacizumab		number randomized = 36
	Placebo		number randomized = 39
	Adolescents		None randomized
Endpoints and definitions	Primary endpoint	Time to confirmed platelet response	Response was defined by a recovery of platelets ≥ 150,000/µL. This response had to be confirmed at 48 hours after the initial reporting of platelet recovery ≥ 150,000/µL by a *de novo* measure of platelets ≥ 150,000/µL and lactate dehydrogenase ≤ 2 x the ULN.
	Secondary endpoint	CR	Defined as confirmed platelet response and absence of exacerbation for 30 days after the last daily PE)
	Secondary endpoint	Exacerbation of aTTP	Defined as recurrent thrombocytopenia following a confirmed platelet response and requiring a re-initiation of daily PE treatment between ≥ 1 day but ≤ 30 days after the last daily PE
	Secondary endpoint	Relapse of aTTP	Defined as *de novo* event of aTTP that occurs later than 30 days after the last daily PE
	Secondary endpoint	Mortality	Within the daily PE treatment period and within the subsequent study drug treatment period (including tapering)
Database lock	<date>		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Caplacizumab	Placebo	
	Number of subject	36	39	
	Median days	2.97	4.79	

	95% CI	2.74, 3.65	3.51, 5.94	

Effect estimate per comparison	Primary endpoint	Comparison groups	
		HR	2.197
		95% CI	1.278, 3.778
		P-value	0.005
	Secondary: CR	Caplacizumab	Placebo
		n (%): 29 (80.6)	n (%): 18 (46.2)
		95% CI: 64.0, 91.8)	95% CI: 30.1, 62.8
		P-value: N/A	P-value N/A
	Secondary: Exacerbations of aTTP	Caplacizumab	Placebo
		n (%): 3 (8.3)	n (%): 11 (28.2)
		95% CI: 1.8, 22.5	95% CI: 15.0, 44.9
		P-value: N/A	P-value: N/A
	Secondary: Relapse of aTTP	Caplacizumab n (%): 11 (30.6) 95% CI: 16.3, 48.1 P-value: N/A	Placebo n (%): 3 (7.7) 95% CI: 1.6, 20.9 P-value: N/A
	Secondary: Mortality	Caplacizumab n=0	Placebo n=2

Title: A Phase III double-blind, randomized, parallel group, multicenter placebo-controlled trial to study the efficacy and safety of caplacizumab in patients with acquired thrombotic thrombocytopenic purpura.		
Study identifier	ALX0681-C301	
Design	A double-blind, randomized, parallel group, multicenter placebo-controlled trial	
	Duration of main phase:	Duration of daily plasma exchange (PE) + 30 days after last daily PE + optional extension for a maximum of 4 additional 1-week periods (i.e., 28 days)
	Duration of Run-in phase:	Not applicable
	Duration of Extension phase:	Treatment-free follow-up of 28 days

Hypothesis	Superiority		
Treatments groups	Caplacizumab		Number randomized = 72
	Placebo		Number randomized = 73
Endpoints and definitions	Primary endpoint	Time to confirmed platelet response	Platelet count response was defined as initial platelet count ≥ 150,000/µL with subsequent stop of daily PE within 5 days. It refers to the first time both conditions, platelet count ≥ 150,000/µL and the stop of daily PE within 5 days., were met.
	First key secondary endpoint	TTP-Related Death, Recurrence of TTP, or a Major Thromboembolic Event	Proportion of subjects with TTP-Related Death, Recurrence of TTP, or a Major Thromboembolic Event During the Study Drug Treatment Period
	Second key secondary endpoint	Recurrence of TTP	Proportion of subjects with a recurrence of TTP in the Overall Study Period
	Third key secondary endpoint	Refractory disease	The proportion of subjects with refractory disease, defined as absence of platelet count doubling after 4 days of standard treatment and lactate dehydrogenase > Upper Limit of Normal
	Fourth key secondary endpoint	Organ damage markers	Time to normalization of organ damage marker levels (Lactate dehydrogenase, cardiac troponin and serum creatinine)
Database lock	20 September 2017		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent-to-treat Only data from the double blind period were used.		
Descriptive statistics and estimate variability	Treatment Group	Caplacizumab	Placebo
	Number of subjects	72	73

	25th Percentile (95% CI) - days	1.75 (1.65; 1.87)	1.94 (1.70; 2.64)
	50th Percentile (95% CI) - days	2.69 (1.89; 2.83)	2.88 (2.68; 3.56)
	75th Percentile (95% CI) - days	2.95 (2.85; 3.81)	4.50 (3.78; 7.79)
	Hazard ratio (95%CI)	1.55 (1.095; 2.195)	
Effect estimate per comparison	Primary endpoint	p-value (stratified log-rank test) = 0.0099	
	First key secondary endpoint: TTP-Related Death, Recurrence of TTP, or a Major Thromboembolic Event	Caplacizumab	Placebo
		Number of subjects assessed: 71	Number of subjects assessed: 73
		Proportion: 12.7%	Proportion: 49.3%
		P-value (CMH test adjusting for Glasgow coma scale [GCS] core at randomization): <0.0001	
	Second key secondary endpoint: Recurrence of TTP	Caplacizumab	Placebo
		Number of subjects assessed: 71	Number of subjects assessed: 73
		Proportion: 12.7%	Proportion: 38.4%
		P-value (CMH test adjusting for GCS at randomization) = 0.0004	
	Third key secondary endpoint: Refractory disease	Caplacizumab	Placebo
		Number of subjects assessed: 71	Number of subjects assessed: 73
		Proportion: 0%	Proportion: 4.2%
		P-value (CMH test adjusting for GCS at randomization) = 0.0572	
	Fourth key secondary endpoint: Organ damage markers	Caplacizumab	Placebo
		Number of subjects assessed: 65	Number of subjects assessed: 66
		Median time - days (95% CI) = 2.86 (1.93; 3.86)	Median time - days (95% CI) = 3.36 (1.88; 7.71)
		P-value: The key secondary endpoints were hierarchically ordered to allow statistical testing for these endpoints at the same nominal significance level of 5% without	

		adjustment, as long as the tests occurred in the pre-defined sequential order, and given that all null hypotheses tested for endpoints with a higher rank (including the primary endpoint) were rejected. No confirmatory testing was done for this fourth key secondary endpoint, as the statistical test was not significant for the proportion of subjects with refractory disease (i.e., the third key secondary endpoint).

Clinical studies in special populations

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	9 / 220 subjects	5 / 220 subjects	0 / 220 subjects
Non Controlled Trials	Not applicable	Not applicable	Not applicable

2.5.2. Discussion on clinical efficacy

Design and conduct of clinical studies

The interpretation of the phase II study results as well as the internal validity of the study must be deemed hampered by several issues related to the conduct: the premature termination of the study, a high number of important protocol amendments, a relatively large fraction of major protocol violations and consequently a small PP population, issues with the local and central laboratories resulting in uncertainties regarding the integrity and identity for an unknown number of the samples, missing data, with the extent sometimes not clear to the assessor, and a number of the analyses considered important analysed post-hoc, and several of the pre-planned and post-hoc ≥secondary outcomes are associated with different levels of uncertainties. Therefore, the data from this study was not considered adequate to be reflected in the product information.

In the ALX0681-C301 study any major protocol deviation was reported for 45% of subjects in the caplacizumab arm and 43% in the placebo arm with “treatment non-compliance” being the most prevalent reason. However, the protocol deviations are not deemed likely to favour the experimental arm or to challenge the overall results of the study.

Efficacy data and additional analyses

Despite the shortcomings associated with the conduct of the phase II study it was considered that proof-of-concept has been shown. However, the observation of equal numbers of exacerbation/relapse at the 1-month follow-up of that study clearly indicates that the optimal use of the drug was not yet established and the limited data on risk-adapted treatment, longer-term treatment and re-treatment upon relapse available so far did not allow firm optimised treatment recommendations. Further, robust data supporting faster resolution of organ damage secondary to microthrombi was largely lacking.

With the submission of the final CSR for the ALX0681-C301 study most of these uncertainties have been addressed. While the allowance of disease activity-guided adaptation of immunosuppression and the possibility for treatment extension were part of the protocol and clearly diminished the risk for recurrence of TTP during the study period in the caplacizumab arm, uncertainties still remain regarding treatment of longer duration than investigated in the study (see RMP).

Further, no data on re-treatment is currently available but this will be addressed in the ongoing ALX0681-C302 study (please see RMP).

2.5.3. Conclusions on the clinical efficacy

The provided efficacy data is deemed sufficient to support a benefit/risk estimation for caplacizumab as adjunct to PE and immunosuppressive therapy in the treatment of adults experiencing an episode of aTTP.

In order to evaluate the long-term efficacy and safety of caplacizumab and the safety and efficacy of repeated use of caplacizumab in the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTPP), the applicant should submit the results of a phase IIIb study (ALX0681-C302).

2.6. Clinical safety

Patient exposure in study ALX-0681-2.1/10 ("TITAN")

		ALX-0081 N=35	Placebo N=37	Total N=72
Overall duration of exposure (days) [1]	n	35	37	72
	Mean	37.9	39.2	38.6
	SD	14.99	18.61	16.85
	Median	36.0	37.0	36.5
	Min, Max	3, 77	2, 90	2, 90
Duration of exposure during daily PE phase (days) [2]	n	35	37	72
	Mean	6.1	8.1	7.1
	SD	2.66	6.50	5.08
	Median	6.0	6.0	6.0
	Min, Max	3, 16	2, 36	2, 36
Duration of exposure post daily PE phase (days) [3]	n	33	33	66
	Mean	33.8	35.0	34.4
	SD	11.98	13.74	12.81
	Median	30.0	30.0	30.0
	Min, Max	4, 70	2, 78	2, 78

Notes:
[1] Duration = last dose date – first dose date + 1
[2] Duration = last dose date during daily PE – first dose date during daily PE + 1
[3] Duration = last dose date – first dose date after daily PE + 1

Adverse events

Table 15: Summary of adverse events in Study ALX-0681-2.1/10 (Safety population)

Description	Caplacizumab N=35		Placebo N=37	
	n*	n (%)	n*	n (%)
Subjects with				
At least one TEAE [1]	574	34 (97.1)	545	37 (100)
At least one study drug-related TEAE [2]	72	20 (57.1)	15	5 (13.5)
At least one TEAE leading to death	0	0	2	2 (5.4)
At least one SAE	44	20 (57.1)	36	19 (51.4)
At least one drug-related SAE	12	7 (20.0)	0	0
At least one TEAE leading to discontinuation of study drug	10	4 (11.4)	2	2 (5.4)
At least one TEAE leading to interruption of study drug	5	3 (8.6)	5	4 (10.8)
At least one TEAE leading to interruption or discontinuation of study drug	15	7 (20.0)	7	6 (16.2)

%=percentage of n based on N; AE=adverse event; N=number of subjects in population; n*=number of events; n=number of subjects with events; SAE=serious TEAE; TEAE=treatment-emergent adverse event

Notes:

[1] A TEAE was defined as an AE with an onset date on or after the first dose of study drug.

[2] A drug-related TEAE was defined as a TEAE which was related or possibly related to study drug.

Table 16: Summary of adverse events by severity in Study ALX-0681-2.1/10 (Safety population)

	Caplacizumab N=35		Placebo N=37	
	n*	n (%)	n*	n (%)
Subjects with any [1]				
Mild TEAE	348	31 (88.6)	299	36 (97.3)
Moderate TEAE	154	27 (77.1)	173	31 (83.8)
Severe TEAE	37	18 (51.4)	23	14 (37.8)

%=percentage of n based on N; AE=adverse event; N=number of subjects in population of interest; n*=number of AEs; n=number of subjects with AEs; TEAE=treatment-emergent adverse event

Notes:

[1] The numbers of TEAEs in this Table does not include events with missing severity (85 cases). As per the Statistical Analyis Plan, any missing severity was left as missing and categorized as such.

A higher frequency of mild to moderate TEAEs was registered in the placebo arm. Severe TEAEs were reported in a higher proportion in the experimental arm. Notably, assessment of severity was missing for 85 cases.

Overall, bleeding-related TEAEs were numerically more common in the caplacizumab arm, with 19 patients (54%) vs. placebo, 14 patients (38%). The mild bleeding events were 80% (53/66) for caplacizumab and 89% (31/35) for placebo. Those moderate in severity were 17% (11/66) for caplacizumab vs 9% (3/35) in the placebo arm.

The most common bleeding-related TEAEs in the caplacizumab treatment group were epistaxis (31%), gingival bleeding (14%), bruising (11%), petechiae (11%), and hematoma (11%), all with mild to moderate severity. The most common PTs in the placebo treatment group were epistaxis and hematoma, both occurring at an incidence of 11%.

With the exception of procedural complications (bruising), 11 vs 5%, and epistaxis (31% vs 11%), other SOC/PTs that were more frequent in the caplacizumab arm were: gynaecological bleeding (11% vs 5%), skin and subcutaneous tissue disorders (17% vs 5%) and haemorrhage/hematoma UNS, 17% vs 11% (including one intra-abdominal hematoma for caplacizumab).

Regarding CNS, one subarachnoid haemorrhage was reported for caplacizumab, and one cerebral haemorrhage (fatal) and one haemorrhagic stroke in the placebo arm.

Two patients experienced a serious bleeding-related TEAE that was considered at least possibly related to caplacizumab (subarachnoid haemorrhage and metrorrhagia, respectively).

The number and proportion of subjects with any immune-related TEAE was higher in the experimental group, 17 patients (49%) compared to control: 12 (32%). Most immune-related TEAEs were mild (43/65 events [66%]) or moderate (20/65 events [31%]) in severity.

One subject in the caplacizumab group experienced 2 non-serious episodes of the TEAE of ‘vaccination site reaction’ during the PE period which were both considered severe in intensity and unlikely/not related to the study drug. In addition, this subject also experienced a serious TEAE of dermatitis allergic during the PE period, which was considered moderate in intensity and possibly related to the study drug and related to PE. No other serious immune-related TEAEs were reported.

Transfusion reaction was more commonly reported in the caplacizumab group: 8 events in 4 patients vs. 1 reaction in 1 subject in the control arm.

Rash and urticarial were reported in similar fractions between study arms.

Clinically relevant bleeding

Most of the clinically relevant bleeding during the first week of daily PE did not require medical or surgical intervention, with the exception of one subject (#602-001) in the caplacizumab treatment group who required medical intervention for metrorrhagia on Days 5 and 6 of daily PE and one subject (#602-006) in the placebo group (Days 18 and 27 of the daily PE period) who required medical intervention (esomeprazole) for vomiting blood on Day 18. The event was reported as mild non-serious AE that resolved without sequelae.

During the 30-day post daily PE period, clinically relevant bleeding was reported in 5 subjects in the caplacizumab treatment group and 2 subjects in the placebo treatment group. Two subjects required medical intervention for bleeding events of epistaxis (#602-003, caplacizumab group) and haematuria (#101-003, placebo group).

No subject in either treatment group had clinically relevant bleeding on Days 3 or 7 or at 1, 2, 3, 6, or 12 months of follow-up.

Serious adverse event / deaths / other significant events

Serious TEAEs were reported in 20 (57.1%) subjects in the caplacizumab treatment group and 19 (51.4%) subjects in the placebo treatment group. Drug-related SAEs were reported in 7 (20%) of caplacizumab patients compared to none in the control arm.

The outcomes of all SAEs that occurred in patients receiving caplacizumab were reported as recovered / resolved (n=19). The outcome of the SAEs that occurred in patients treated with placebo were reported as recovered/resolved (n=17) and as fatal (n=2).

No TEAEs led to death in the experimental group, while 2 TEAEs resulted in death in the placebo arm, both occurring between the end of study drug treatment up to and including 1 month follow-up.

Laboratory findings

Mean fibrinogen, aPTT levels, PT levels, and the INR were normal at baseline, on the first day after daily PE and at 1, 6, and 12 months follow-up and were similar in both treatment groups. A post-hoc analysis of transaminases abnormalities showed no major differences between study arms. Please refer to the PK/PD section for a discussion of vWF:Ag and FVIII:C.

Safety in special populations

The age of subjects enrolled ranged from 19 to 72 years with a mean of 42 years. Insufficient data are available to determine a difference in response in patients over 65 years of age compared to the younger patient population. No data in paediatric/adolescent patients (12-18 years) are available.

No patients with severe acute or chronic renal and/or hepatic impairment were studied.

Immunological events

Pre-existing antibodies were detected pre-dose in 13% of the subjects in study ALX-0681-2.1/10. Treatment-emergent anti-drug antibodies (TE ADA) were detected in 9% of the subjects in the caplacizumab treated group and no TE ADA was detected in the placebo treated subjects. It is agreed with the Applicant that the immunogenicity in the caplacizumab treated group (pre-Ab and/or TE ADA)

had no apparent influence on immune-related adverse events, but numbers of subjects are low and aTTP a complex disease. Please also refer to the PK/PD section.

Safety related to drug-drug interactions and other interactions

Ongoing chronic treatment with anticoagulant treatment that could not be stopped safely was an exclusion criterion.

Discontinuation due to adverse events

AEs leading to discontinuation of study drug were numerically higher in the caplacizumab arm, 10 events in 4 subjects as compared to 2 events in 2 subjects in the control arm. No contributing specific PT can be identified to be responsible for the difference.

AEs leading to interruption of study drug were similar between study arms and noted in 3 patients in the caplacizumab arm and 4 patients in the control arm.

Study ALX681-C301

Exposure and overview

Total treatment duration during the DB period was 35 days in the caplacizumab arm and 23 days in the placebo arm. This difference should be taken into account when looking at incidence of events. During the OL treatment Period, the median duration of OL caplacizumab treatment was 36 days.

Table 17: Summary table of treatment-emergent adverse events during the Overall Study Period (Safety population)

Number of subjects with, n (%)	Double-Blind Caplacizumab (N = 71)	Double-Blind Placebo (N = 73)
At least one TEAE	69 (97.2)	71 (97.3)
At least one SAE	28 (39.4)	39 (53.4)
At least one TEAE leading to death	1 (1.4)	3 (4.1)
At least one TEAE for which the study drug was withdrawn	5 (7.0)	9 (12.3)
At least one TEAE that was considered at least possibly treatment-related	41 (57.7)	32 (43.8)
At least one SAE that was considered at least possibly treatment-related	10 (14.1)	4 (5.5)
At least one bleeding event (SMQ "Haemorrhage")	49 (69.0)	49 (67.1)
At least one bleeding event (CRF documented event with increased bleeding tendency)	47 (66.2)	36 (49.3)

Abbreviations: N = total number of subjects in treatment group; n = number of subjects with events; CRF = case report form; SAE = serious adverse event; SMQ = Standardised MedDRA Query; TEAE = treatment-emergent adverse event.
Note: Percentage was calculated using the number of subjects in the Safety population as the denominator.

During the overall study period, SAEs were reported in 39% of subjects in the caplacizumab arm and 53% of subjects in the placebo arm with 14% and 6%, respectively, considered at least possibly treatment-related; TEAEs leading to study drug withdrawal were reported in 7% and 12%, respectively. The frequency of bleeding events considered indicative of an increased bleeding tendency was higher in the caplacizumab arm. Note that the figures for bleeding events according to SMQ “haemorrhage” are confounded by TTP events.

Adverse events (overall study period)

By preferred term, the most frequently reported TEAEs were:

• Thrombotic thrombocytopenic purpura reported in 9 subjects (12.7%) and 29 subjects (39.7%) in the DB caplacizumab and DB placebo groups, respectively.

• Epistaxis reported in 23 subjects (32.4%) and 2 subjects (2.7%) in the DB caplacizumab and DB placebo groups, respectively.

• Headache reported in 16 subjects (22.5%) and 6 subjects (8.2%) in the DB caplacizumab and DB placebo groups, respectively.

TEAEs reported with a higher risk of occurring after treatment with caplacizumab compared to placebo were gingival bleeding, epistaxis, and headache.

TEAEs reported with a lower risk of occurring after treatment with caplacizumab compared to placebo were TTP and hypokalaemia.

During the overall study period severe TEAEs were more commonly reported in the placebo group, 33% vs 21%. The incidences of moderate headache (7% vs 3%) and epistaxis (7% vs 0%) were higher in the caplacizumab group, as was the SOC of infections and infestations (13% vs 7%).

Consistent with the findings in the phase II study, the safety profile of caplacizumab seems to be dominated by mucocutaneous bleeding.

During the overall study period, removing events of TTP and microangiopathy, treatment-emergent SAEs were reported in 19 (27%) subjects in the caplacizumab arm and 9 (12%) patients in the placebo arm. The higher frequency of these SAEs in the caplacizumab arm seems to be mainly related to bleeding events but also a slightly higher fraction in the cardiac disorders SOC is noted. The latter was addressed in Q21: collectively, the PTs and their temporal occurrence are not suggestive of a specific pattern or a clear relationship with caplacizumab treatment. The reasons for the imbalance between study arms remain obscure.

During the overall study period, excluding TTP as event, 6% of subjects in the caplacizumab arm and 10% in the placebo arm discontinued study drug due to AEs; no PT occurred in more than one of these patients.

During the overall study period, treatment-related serious bleeding events were reported for 8 subjects (11%) in the DB caplacizumab group and 3 subjects (4%) in the DB placebo group; epistaxis was reported in 4 subjects (6%) in the DB caplacizumab group and 0 subjects in the DB placebo group.

In the placebo arm, 3 patients died during the daily PE period, all considered TTP-related. In the caplacizumab arm, one patient died during FU, also considered TTP-related.

During the overall study period, at least one hypersensitivity reaction was reported in similar fractions of the study groups. There were no treatment-related events of drug-induced anaphylaxis in the DB caplacizumab group.

If removing TTP and thrombotic microangiopathy, no major difference between groups in terms of treatment-emergent thromboembolic events is noted.

2.6.1. Discussion on clinical safety

In the phase II study, overall, bleeding-related TEAEs were numerically more common in the caplacizumab arm, with 19 patients (54%) vs. placebo, 14 patients (38%). Hence, warnings in section 4.4 of the SmPC have been introduced to recommend that Cablivi treatment should be interrupted in case of active, clinically significant bleeding. If needed, the use of von Willebrand Factor concentrate

could be considered to correct haemostasis. Cablivi should only be restarted upon the advice of a physician experienced in the management of thrombotic microangiopathies.

In addition, warnings have been introduced to provide recommendations when patients are at an increased risk of bleeding (please see section 4.4 of the SmPC), the patient alert card will also provide recommendations to this effect (please see RMP).

The most common adverse reactions were bleeding events: gingival bleeding, epistaxis, Eye Haemorrhage, haematemesis, haematochezia, melaena, upper gastrointestinal haemorrhage, haemorrhoidal haemorrhage, rectal haemorrhage, abdominal wall haematoma, Injection site haemorrhage, subarachnoid haemorrhage, haematuria, menorrhagia, vaginal haemorrhage, haemoptysis, haematoma.

Other most common adverse reactions were pyrexia, fatigue, headache, urticaria, injection site reaction, myalgia, injection site pruritus, injection site erythema, cerebral infarction, dyspnoea.

The mild bleeding events were 80% (53/66) for caplacizumab and 89% (31/35) for placebo. Those moderate in severity were 17% (11/66) for caplacizumab vs 9% (3/35) in the placebo arm. It is noted that assessment of severity was missing for 85 cases. Two patients experienced a serious bleeding-related TEAE that was considered at least possibly related to caplacizumab (subarachnoid haemorrhage and metrorrhagia, respectively). Serious TEAEs were reported in similar fractions of subjects between study arms. The number and proportion of subjects with any immune-related TEAE was higher in the experimental group. AEs leading to discontinuation of study drug were numerically higher in the caplacizumab arm, 10 events in 4 subjects as compared to 2 events in 2 subjects in the control arm. No contributing specific PT can be identified to be responsible for the difference. No patient on caplacizumab died on treatment. Caplacizumab treatment lowers vWF:Ag levels and FVIII:C but no factor concentrates had to be used in the study.

The safety results also from the ALX0681-C301 study are generally compatible with what could be expected from the MoA and mainly consists of von Willebrand disease-like mild to moderate mucosal and skin/subcutaneous tissue haemorrhage.

During the overall study period, treatment-related serious bleeding events were reported for 8 subjects (11%) in the DB caplacizumab group and 3 subjects (4%) in the DB placebo group; epistaxis was reported in 4 subjects (6%) in the DB caplacizumab group and 0 subjects in the DB placebo group. During the overall study period severe TEAEs were more commonly reported in the placebo group. The incidences of moderate headache and epistaxis were higher in the caplacizumab group, as was the SOC of infections and infestations.

During the overall study period, excluding TTP as event, 6% of subjects in the caplacizumab arm and 10% in the placebo arm discontinued study drug due to AEs; no PT occurred in more than one of these patients.

At least one hypersensitivity reaction was reported in similar fractions of the study groups. There were no treatment-related events of drug-induced anaphylaxis in the DB caplacizumab group.

Based on subgroup analyses, no particular risk or safety concern was associated with extending treatment. The number and nature of adverse events was generally similar in patients with or without treatment extension.

No data on re-treatment is currently available but this will be addressed in the ongoing ALX0681-C302 study (please see RMP). In order to evaluate the long-term safety and efficacy of caplacizumab and the safety and efficacy of repeated use of caplacizumab in the treatment of adults experiencing an episode

of acquired thrombotic thrombocytopenic purpura (aTPP), the MAH should submit the results of a phase IIIb study (ALX0681-C302).

2.6.2. Conclusions on the clinical safety

The major safety issue noted with caplacizumab is von Willebrand disease-like mild to moderate mucosal and skin/subcutaneous tissue haemorrhage, in accordance with the MoA. Clinically relevant bleeding was more commonly reported in the caplacizumab arm, this has been reflected in section 4.4 of the SmPC where warning have been introduced and a patient alert card will be given to patients (please see RMP).

No data on re-treatment is currently available but this will be addressed in the ongoing ALX0681-C302 study (please see RMP).

Overall, the safety profile of the drug seems generally manageable.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Bleeding
Important potential risks	Serious hypersensitivity reactions
Missing information	Use in pregnancy and lactation Use in patients with severe hepatic impairment Long term exposure, including immunogenicity

Pharmacovigilance plan

Study Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 3 – Required additional pharmacovigilance activities				
ALX0681-C302 Prospective Follow-up Study for Patients who Completed Study ALX0681-C301 (HERCULES) to Evaluate Long-term Safety and Efficacy of caplacizumab (Post-HERCULES) *Status: Ongoing*	• To evaluate long-term safety and efficacy of caplacizumab • To evaluate safety and efficacy of repeated use of caplacizumab • To characterise long term impact of TTP	Long term safety of caplacizumab. Safety of the repeated use of caplacizumab. Immunogenicity	Final CSR	31/12/2022

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks		
Bleeding	Routine risk minimisation measures: • SmPC section 4.4, 4.8. and 4.9 • PIL section 2 and 4 Legal status: • subject to medical prescription Additional risk minimisation measures: • Patient Alert Card	Routine pharmacovigilance. Enhanced PV activities: • Targeted Questionnaire to follow-up serious events of bleeding until renewal of the MA. Other types of routine PV: • Discussion of risks of bleeding reactions in PSURs Additional pharmacovigilance activities: • None
Important potential risks		
Serious Hypersensitivity reactions	Routine risk minimisation measures: • SmPC sections 4.3 • PIL section 2 and 4 Legal status: • subject to medical prescription Additional risk minimisation measures: • None	Routine pharmacovigilance. Enhanced PV activities: • Targeted Questionnaire to follow-up events of serious hypersensitivity reactions until renewal of the MA. Other types of routine PV: • Discussion of risks of serious hypersensitivity reactions in PSURs Additional pharmacovigilance activities: • ALX0681-C302 (HERCULES Follow-up study). Collect data on repeated use and associated hypersensitivity reactions.
Missing information		
Use in pregnancy and lactation	Routine risk minimisation measures: • SmPC section 4.6 • PIL section 2 Legal status: • subject to medical prescription Additional risk minimisation measures: • None	Routine pharmacovigilance. Reports of exposure during pregnancy and lactation will be followed up using forms designed for this purpose. Additional pharmacovigilance activities: • None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Use in patients with severe hepatic impairment	Routine risk minimisation measures: • SmPC section 4.2 and 4.4 • PIL section 2 Legal status: • subject to medical prescription Additional risk minimisation measures: • None	Routine pharmacovigilance. Enhanced PV activities: • Targeted Questionnaire to follow-up events of bleeding is designed to collect liver function status data. Other types of routine PV: • Discussion of risk of bleeding in PSUR to include available data on liver function status in cases of bleeding. Additional pharmacovigilance activities: • None
Long term exposure, including immunogenicity	Routine risk minimisation measures: • SmPC section 5.1 Legal status: • subject to medical prescription. Additional risk minimisation measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • Discussion in PSURs Additional pharmacovigilance activities: • ALX0681-C302 Long term HERCULES follow-up study. Designed to collect data on long term (3y) safety.

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.6 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfills the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The EBD corresponds to the international birth date (IBD) which will be used to determine the forthcoming Data Lock Points of the new EURD list entry.

2.9. New Active Substance

The applicant declared that caplacizumab has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers caplacizumab to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Cablivi (caplacizumab) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU and it is a biological product that is not covered by the previous category and authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Cablivi is indicated for the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTTP), in conjunction with plasma exchange and immunosuppression.

3.1.2. Available therapies and unmet medical need

With current standard therapy, including PE and corticosteroids, and more recently at certain centres incorporating also rituximab, an acute mortality of 20% has been reported. Refractoriness to therapy is associated with inferior prognosis and increased mortality.

3.1.3. Main clinical studies

Study ALX681-C301: A Phase III double-blind, randomized, parallel group, multicenter placebo-controlled trial to study the efficacy and safety of caplacizumab in patients with acquired thrombotic thrombocytopenic purpura.

3.2. Favourable effects

Study ALX681-C301

Primary endpoint; time to confirmed platelet response

Based on 66 events in each arm time to confirmed platelet response was statistically significantly shorter in the caplacizumab arm, p=0.0099, with a HR of 1.55 (1.095; 2.195). In terms of medians, the absolute outcomes as well as the difference between study arms were much smaller than anticipated in the sample size calculations, only 0.19 days (2.69 vs 2.88). However, looking at the KM graph the median is not informative. Rather, the effect of caplacizumab is noted primarily in "late" responders, >3-4 days. Opposed to the phase II study, patients were required to have received one PE treatment before randomization, which likely contributed to the shorter times to platelet response observed in the phase III study. The result of the primary analysis is supported by various sensitivity analyses. The subgroup analyses, addressing HR for time to platelet count response in relation severity of disease, initial/recurrent TTP episode and ADAMTS13 level show generally consistent treatment effects.

Treatment with caplacizumab resulted in a 74% reduction in the composite endpoint of the percentage of patients with aTTP-related death (0/72; placebo 3/73), exacerbation of aTTP (3/72; placebo 28/73), or at least one major thromboembolic event during study drug treatment (6/72; placebo 6/73) (p<0.0001). There were no deaths in the caplacizumab group and 3 deaths in the placebo group during the study drug treatment period.

Treatment with caplacizumab reduced the mean number of days of plasma exchange, the volume of plasma used, the mean length of Intensive Care Unit stay and the mean length of hospitalization during the study drug treatment period (see effects table).

The results of the ALX681-C301 study also emphasise the need for monitoring (e.g. ADAMTS13 activity) and further immunosuppressive treatment of persistent disease activity (please see section 4.2 of the SmPC).

3.3. Uncertainties and limitations about favourable effects

Data from study ALX-0681-2.1/10 were not considered adequate to be reflected in the product information due to limitations with regards to the conduct of the study.

Uncertainties still remain regarding the benefit-risk in treatment of longer duration than investigated in the study. Further, no data on re-treatment is currently available but will be addressed in the ongoing ALX0681-C302 study. Hence, in order to evaluate the long-term efficacy and safety of caplacizumab and the safety and efficacy of repeated use of caplacizumab in the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTPP), the MAH should submit the results of a phase IIIb study (ALX0681-C302) (see RMP).

3.4. Unfavourable effects

The most common adverse reactions were bleeding events: gingival bleeding, epistaxis, eye haemorrhage, haematemesis, haematochezia, melaena, upper gastrointestinal haemorrhage, haemorrhoidal haemorrhage, rectal haemorrhage, abdominal wall haematoma, Injection site haemorrhage, subarachnoid haemorrhage, haematuria, menorrhagia, vaginal haemorrhage, haemoptysis, haematoma.

Hence, warnings in section 4.4 of the SmPC have been introduced to provide recommendation that Cablivi treatment should be interrupted case of active, clinically significant bleeding. If needed, the use of von Willebrand Factor concentrate could be considered to correct haemostasis. Cablivi should only

be restarted upon the advice of a physician experienced in the management of thrombotic microangiopathies.

In addition, warnings have been introduced to provide recommendations when patients are at an increased risk of bleeding (please see section 4.4 of the SmPC) and a patient alert card will also provide recommendations to this effect.

Other most common adverse reactions were pyrexia, fatigue, headache, urticaria, injection site reaction, myalgia, injection site pruritus, injection site erythema, cerebral infarction, dyspnoea.

TE ADA responses and TE Nab detection were noted at higher frequencies in the caplacizumab group as compared to placebo, but absolute numbers were low. No influence of pre-Ab or TE ADA on time to platelet count response was found. No TE ADA were detected in any of the 3 subjects with exacerbation in the caplacizumab arm.

3.5. Uncertainties and limitations about unfavourable effects

Uncertainties still remain regarding the safety of longer duration than investigated in the studies, as well as due to the relatively small size of the safety database in the target population (n=72 in the ALX681-C301 study and 35 in the ALX-0681-2.1/10 study). Hence, in order to evaluate the long-term efficacy and safety of caplacizumab and the safety and efficacy of repeated use of caplacizumab in the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTPP), the MAH should submit the results of a phase IIIb study (ALX0681-C302). The 3 years follow-up study will be informative for further characterisation of the safety profile of caplacizumab (see RMP).

3.6. Effects Table

Table 18: Effects Table for Cablivi (data cut-off: 20 September 2017):

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
			N=72	N=73		
time to platelet count response	Defined as initial platelet count ≥ 150×109/L with subsequent stop of daily PE within 5 days.	Platelet count	HR=1.55 (1.095; 2.195)		P value=0.0099	Clinical efficacy - Study ALX0681-C301
Proportion of subjects with a recurrence of TTP in the overall study period:		%	13	38	P value=0.0004	Clinical efficacy - Study ALX0681-C301
Number of days of plasma exchange		days	5.8 (0.51)	9.4 (0.81)		Clinical efficacy - Study ALX0681-C301
Total volume of plasma		Litre	21.33 (1.62)	35.93 (4.17)		Clinical efficacy - Study

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
used						ALX0681-C301
Lengths of hospitalisations		Days	9.9 (0.70)	14.4 (1.22)		Clinical efficacy - Study ALX0681-C301
Number of days in ICU		Days	3.4 (0.4)	9.7 (2.12)		Clinical efficacy - Study ALX0681-C301
Unfavourable Effects						
Bleeding events	SMQ "haemorrhage"	Number of patients	49 (69.0)	49 (67.1)		Clinical safety - Study ALX0681-C301
Bleeding event	CRF documented event with increased bleeding tendency	Number of patients	47 (66.2)	36 (49.3)		Clinical safety - Study ALX0681-C301
TEAE considered possibly treatment related		Number of patients	41 (57.7)	32 (43.8)		Clinical safety - Study ALX0681-C301
Deaths		Number of patients	1	3	Deaths were considered TTP-related.	Clinical safety - Study ALX0681-C301

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Data are deemed sufficiently robust to support a B/R estimation for caplacizumab as adjunct to PE and immunosuppressive therapy in the treatment of adults experiencing an episode of aTTP.

With the addition of caplacizumab to current standard of care the substantial reduction in the risk for aTTP recurrence, noted also during the overall study period, is, for obvious reasons, deemed to constitute an important clinical outcome. However, concomitant monitoring, e.g. for ADAMTS13 activity, and optimized immunosuppressive treatment of persistent disease activity are prerequisites for optimal use of the drug.

The phase III study failed to show superiority in terms of time to normalisation of the 3 selected tissue markers, although the median time was numerically shorter in the caplacizumab arm, and no difference in terms of treatment-emergent major thromboembolic events was noted between study arms. Nevertheless, a shorter time to platelet response is considered of clinical relevance as it reduces the time at the highest risk for morbidity and leads to shorter primary exposure to PE and the procedure-associated risks.

The clinical relevance of the two efficacy outcomes discussed above, reduction in the risk for aTTP recurrence and shorter time to platelet response, is supported by numerically shorter median number of days in ICU and hospital, shorter median number of days of PE and lower absolute total volume of PE, all during the overall study period, in the phase III caplacizumab arm. Looking at the DB daily PE period, 3 patients died in the placebo group and none in the caplacizumab group, all considered TTP-related.

The most important safety issue noted with caplacizumab is von Willebrand disease-like mild to moderate mucosal and skin/subcutaneous tissue haemorrhage, in accordance with the MoA. Although relatively uncommon, clinically relevant bleeding was noted with caplacizumab. Generally, the safety profile of the drug is deemed manageable.

In order to evaluate the long-term efficacy and safety of caplacizumab and the safety and efficacy of repeated use of caplacizumab in the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTPP), the MAH should submit the results of a phase IIIb study (ALX0681-C302) (please see RMP).

3.7.2. Balance of benefits and risks

The benefits are considered to outweigh the risks.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall B/R of Cablivi is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Cablivi is favourable in the following indication:

Cablivi is indicated for the treatment of adults experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTTP), in conjunction with plasma exchange and immunosuppression.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Cablivi in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the patient alert card, including communication media, distribution modalities, and any other aspects, with the National Competent Authority.

The MAH shall ensure that in each Member State where Cablivi is marketed, all patients/carers who are expected to use Cablivi are provided with the following patient alert card which shall contain the following key message:

- to mitigate the risk of serious bleeding episode particularly in emergency situations (e.g. accident)
- to inform physicians about the medical blockage of the von Willebrand Factor.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that caplacizumab is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.