CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761112Orig1s000

CHEMISTRY REVIEW(S)

Recommendation: Approval

BLA Number: 761112
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Review Number: First Round
Review Date: December 21, 2018

Drug Name/Dosage Form	Cablivi (caplacizumab-yhdp[1]), for injection
Strength/Potency	(b) (4) (11 mg nominal dose[2] after reconstitution in water using provided pre-filled syringe)
Route of Administration	intravenous and subcutaneous
Rx/OTC dispensed	Rx
Indication	CABLIVI is indicated for the treatment of adult patients with acquired thrombotic thrombocytopenic purpura (aTTP), in combination with plasma exchange and corticosteroid therapy.
Applicant/Sponsor	Ablynx NV

Product Overview

Caplacizumab-yhdp is a bivalent single domain heavy chain antibody fragment (referred to as "nanobody[3]" variable domain dimer by the applicant) produced in *E. coli* that consists of two identical humanized building blocks (denominated PMP12A2h1) that are covalently linked by a three-alanine linker; it consists of 259 amino acids and has a molecular weight of 27876 Dalton. These single domain antibody fragments represent a novel therapeutic class of proteins derived from the heavy chain variable domains that occur naturally in heavy chain-only immunoglobulins from llamas and other *camelidae* species; they have a high degree of homology to human immunoglobulin heavy chain variable region domains.

Caplacizumab-yhdp is directed towards the human A1 domain of von Willebrand factor (vWF) for the treatment of aTTP, which has orphan drug designation status in the USA (FDA letter dated April 14, 2009).

Quality Review Team

Discipline	Reviewer	Center/Office/Division
Drug Substance, Drug Product	Jacek Cieslak	CDER/OPQ/OBP/DBRR IV
Immunogenicity Assays Validation	Leopold Kong	CDER/OPQ/OBP/DBRR IV
Labeling	CAPT Vicky Borders Hemphill	CDER/OPQ/OBP
Facilities	Viviana Matta	CDER/OPQ/OPF/DIA
Microbiology	Scott Nichols Candace Gomez-Broughton	CDER/OPQ/OPF/DMA IV
Device (combination product)	Florencia Wilson	CDRH/ODE/DAGRID/GHDB
Team Leads	LCDR Leslie A. Rivera Rosado (Product quality) Peter Qiu (Facilities)	CDER/OPQ/OBP/DBRR IV CDER/OPQ/OPF/DIA

[1] The nonproprietary name, caplacizumab-yhdp, was conditionally accepted on 11/20/2018 by the FDA.
[2] The applicant proposes that the nominal dose after reconstitution is 10 mg. However, based on data provided by the applicant, OPQ recommends that the product be labeled with the actual dose of 11 mg (refer to section E of this memo).
[3] "Nanobody" is a registered trademark of Ablynx N.V.

Discipline	Reviewer	Center/Office/Division
	Reyes Candau-Chacon (Microbiology) Carolyn Dorgan (Device)	CDER/OPQ/OPF/DMA IV CDRH/ODE/DAGRID/GHDB
OPQ RBPM	Melinda Bauerlien	CDER/OPQ/OPRO
Application Team Lead	LCDR Leslie A. Rivera Rosado	CDER/OPQ/OBP/DBRR IV

Multidisciplinary Review Team

Discipline	Reviewer	Center/Office/Division
RPM	Beatrice Kallungal and Laura Wall	CDER/OND/OHOP/DHP
Cross-disciplinary Team Lead	Kathy Robie Suh	CDER/OND/OHOP/DHP
Medical Officer	Andrew Dmytrijuk	CDER/OND/OHOP/DHP
Clinical Safety	Virginia Kwitkowski	CDER/OND/OHOP/DHP
Pharm/Tox	Brenda Gehrke Christopher Sheth	CDER/OND/OHOP/DHOT
Clinical Pharmacology Pharmacometrics/PBPK	Robert Schuck Olanrewaju Okusanya Justin C Earp Qiu, Junshan	CDER/OTS/OCP/DCPV CDER/OTS/OCP/DCPV CDER/OTS/OCP/DPM CDER/OTS/OCP/DPM
Statistics	Kunthel By Yuan Li Shen	CDER/OTS/OB/DBV
Pharmacovigilance	Regina Lee Peter Waldron	CDER/OSE/OPE/DPVI I
Epidemiology	Carolyn McCloskey	CDER/OSE/OPE/DEPI I
Medication Error Prevention and Analysis	Nicole Garrison Hina Mehta	CDER/OSE/OMEPRM/DMEPA
Risk Management	Naomi Redd Elizabeth Everhart	CDER/OSE/OMEPRM/DRISK

Names:

Proprietary Name:	Cablivi (cab-LIV-ee)
Non-proprietary/USAN/INN:	caplacizumab-yhdp
CAS Registry Number:	915810-67-2
Chemical Name:	Immunoglobulin, anti-(human von Willebrand's blood coagulation factor VIII domain A1) (human-*Lama glama* dimeric heavy chain fragment PMP12A2h1)
Company Name:	ALX-0081
OBP systematic name[4]:	MABFRAG HUMANIZED BIVALENT ANTI P04275 (VWF_HUMAN) [ALX0081]

Submissions Reviewed:

Submission	Date Received	Review Completed by:
STN 761112/1- BLA Original Application	4/4/2018	All
STN 761112/2- Response to 4/17/2018 IR	4/25/2018	OPF
STN 761112/3- Update to response to 4/17/2018 IR	4/27/2018	OPF

[4] The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the nonproprietary name. The tag at the end is used to separate products from different sponsors and it is generally the name used by sponsors to refer to the proposed product in their submissions.

Submission	Date Received	Review Completed by:
STN 761112/4- Rolling submission part 2	6/6/2018	All
STN 761112/7- Response to 7/10/2018 IR	7/13/2018	OPF
STN 761112/10- Labeling/PI draft	8/2/2018	OBP Labeling
STN 761112/11- Response to 8/7/2018 IR	8/22/2018	OPF/DMA
STN 761112/12- Response to 9/14/2018 IR	9/24/2018	OBP/CDRH device
STN 761112/15- Response to 9/20/2018 IR	10/5/2018	OPF/DMA
STN 761112/17- Response to 10/11/2018 IR	10/19/2018	OPF/DMA
STN 761112/18- Response to 9/20/2018 IR	10/23/2018	OPF/DMA
STN 761112/19- Response to 8/7/2018 IR	11/2/2018	OPF/DMA
STN 761112/20- Response to 10/13/2018 IR	11/2/2018	OBP
STN 761112/21- Response to 10/31/2018 IR	11/13/2018	OBP
STN 761112/22- Response to 11/8/2018 IR	11/14/2018	OBP (Immuno assays)
STN 761112/23- Response to 10/31/2018 & 11/5/2018 IRs	11/16/2018	OPF/DMA
STN 761112/24- Response to 11/16/2018 IR	11/21/2018	OBP
STN 761112/25- Response to 10/31/2018 IR	11/26/2018	CDRH
STN 761112/26- Response to 11/21/2018 IR	11/27/2018	OPF/DMA, CDRH
STN 761112/27- Response to 10/31/2018, 11/5/2018, 11/16/2018 and 11/21/2018 IRs	11/30/2018	OBP, OPF/DMA
STN 761112/28- Response to 12/04/2018	12/10/2018	OBP, OPF/DMA

Quality Review Data Sheet

1. Legal Basis for Submission: 351(a)

2. Related/Supporting Documents

A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Code[a]	Status[b]	Date Review Completed	Comments
(b) (4)	III	(b) (4)		3	N/A	N/A	There is enough data in the application; therefore, the DMFs did not need to be reviewed.
	III			3	N/A	N/A	
	III			3	N/A	N/A	

a. Action codes for DMF Table: 1- DMF Reviewed; Other codes indicate why the DMF was not reviewed, as follows: 2- Reviewed previously and no revision since last review; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under "comments")
b. Adequate, Adequate with Information Request, Deficient, or N/A (There is enough data in the application; therefore, the DMF did not need to be reviewed).

B. Other documents: IND 107609

3. Consults:

Discipline/Topic	Date Requested	Status	Recommendation	Reviewer
CDRH/technical/ engineering Review of delivery device	8/13/2018	Completed 12/6/2018	The device constituent of the combination product is **approvable** for the proposed indication.	CDRH-ODE: Florencia Wilson Carolyn Dorgan (TL)
CDRH/Facilities inspections	8/13/2018	Completed 12/6/2018	CDRH does not need to conduct a compliance evaluation of the application.	CDRH-OC: M. Isabel Tejero del Rio CDRH-ODE: Florencia Wilson

Executive Summary

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

The Office of Pharmaceutical Quality (OPQ), CDER, has completed review of STN 761112 for Cablivi (caplacizumab-yhdp) manufactured by Ablynx NV. The data submitted in this application are adequate to support the conclusion that the manufacture of Cablivi is well-controlled and leads to a product that is pure and potent. OPQ recommends that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

Manufacturing locations:

- Drug Substance:
  - o (b) (4)
- Drug Product:
  - o (b) (4)
- Solvent:
  - o (b) (4)

Fill size and dosage form:

- 11 mg in a single-dose vial for injection[5]

Dating period:

- Drug Substance: (b) (4) months at ≤ (b) (4) °C
- Drug Product: 48 months at 2°C – 8°C
- Solvent: (b) (4) months at (b) (4)

[5] The applicant proposes that the nominal dose after reconstitution is 10 mg. However, based on data provided by the applicant, OPQ recommends that the product be labeled with the actual dose of 11 mg (refer to section E of this memo).

- Packaged products (kit):
  - Drug Product Vial: (b) (4) months at (b) (4)
  - Solvent: months at
  - Vial adapter: months
  - Needle: months
  - Alcohol swabs: months

The expiration date of each kit is determined by the expiration date of the component expiring first and will be printed in MMM YYYY format on the secondary packaging, the vial, and the syringe label.

Exempt from lot release:

- Yes, Cablivi is a specified product exempted according to 21 CFR 601.2a.

C. Benefit/Risk Considerations:

The assessment of manufacturing information provided in the application has concluded that the methodologies and processes used for drug substance and drug product manufacturing, release and stability testing are robust and sufficiently controlled to result in a consistent and safe product. The drug substance manufacturing process is robust for (b) (4) No approvability issues were identified from a sterility assurance or microbiology product quality perspective.

The caplacizumab-yhdp drug substance (DS) will be manufactured at (b) (4) the Cablivi drug product (DP) at (b) (4) and the pre-filled syringe with water for injection (WFI; solvent) will be manufactured at (b) (4) Quality control testing will be performed mostly at Ablynx NV (FEI: 3008915297) and at other sites as summarized in section E of this document. Pre-licensing inspections were conducted at (b) (4) A surveillance inspection of the (b) (4) All facilities used for the manufacture and quality control testing of the combination product were found acceptable for the proposed operations (refer to sections E and F of this document).

CDRH Office of Compliance and Office of Device Evaluation provided evaluations of the device component of the product; no approvability issues were identified from a device component perspective.

The immunogenicity assays are sufficiently sensitive to detect anti-drug antibodies (ADA) in presence of caplacizumab-yhdp at plasma concentrations.

Individual assessments for each discipline, (1) drug substance and drug product quality [OBP], (2) immunogenicity assays [OBP], (3) microbiological control of drug substance and drug product [DMA], (4) facilities evaluation [DIA], (5) device evaluation [CDRH/ODE] and (6) labeling [OBP] are located as separate documents in Panorama.

D. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable:

Draft language:

a. To validate shipping of bulk drug substance from [redacted] (b) (4) Italy during summer conditions.

b. To conduct a study to demonstrate that the pre-filled syringe plunger movement during transport does not impact product sterility.

c. To repeat the [redacted] (b) (4) bacterial retention study using a non-bactericidal surrogate solution with physical attributes comparable to the product.

d. To develop and implement an analytical method to determine polysorbate 80 (PS80) levels as [redacted] (b) (4) control on ALX-0081 drug product (DP) in-process samples with appropriate upper and lower limits. The final report, including method validation reports and assessment of the acceptance criterion for polysorbate 80 based on method capabilities and available data, should be submitted as a Prior Approval Supplement.

e. To perform the testing for resistance to overriding for sWFI syringe in accordance to ISO-80369-7 and submit the testing report.

II. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1 is a summary of product-related critical quality attributes (CQA), intrinsic to the molecule, that are relevant to both drug substance (DS) and drug product (DP). The table includes the identification of the various attributes along with their risk management.

Table 1: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA type	CQA	Risk[6]	Origin	Control Strategy
Potency (biological activity)	[redacted] (b) (4)	PK/PD, Biological activity, safety	Intrinsic to the molecule	[redacted] (b) (4)
Identity	Identity	Biological activity, PK/PD, safety	Intrinsic to the molecule	
Product-related impurities	High Molecular Weight (HMW) species	Theoretical impact to PK/PD, biological activity, immunogenicity, safety	Only trace amounts observed. No increase under accelerated or stressed temperature storage conditions.	

[6] Based on the risk assessment provided by the applicant in section 3.2.S.2.6 Manufacturing Process Development Attachment 16 of the BLA submission.

CQA type	CQA	Risk[6]	Origin	Control Strategy
			Small increase under (b) (4)	
	Low Molecular Weight (LMW) species	Theoretical impact to PK/PD, biological activity, immunogenicity, safety	Only trace amounts observed. No increase under accelerated or stressed temperature storage conditions. Small increase under (b) (4)	(b) (4)
	(b) (4)	Safety, Biological activity (loss of potency), potential impact on PK/PD	Mainly formed in DS, during storage at higher temperatures.	
		unknown	Cell culture	
Non-CQAs controlled to ensure process consistency				
(b) (4)				

DS - Drug Substance, DP - Drug Product, CQA – Critical Quality Attributes, (b) (4)

PK – Pharmacokinetics, PD – Pharmacodynamics

B. Drug Substance, caplacizumab-yhdp, Quality Summary

Table 2 provides a summary of the identification, risk, and lifecycle knowledge management for drug substance CQAs that derive from the drug substance manufacturing process and general drug substance attributes, including process-related impurities.

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.

CQA type	CQA	Risk	Origin	Control Strategy
Process-related impurities	Host Cell DNA	Safety	Cell culture	(b) (4)
	Host Cell Proteins (HCP)	Safety (Immunogenicity)	Cell culture	
	Leachables	Safety (Immunogenicity)	Raw materials, product-contacting equipment and materials	

CQA type	CQA	Risk	Origin	Control Strategy
				(b) (4)
	(b) (4)	Low safety risk. Unknown immunogenicity risk.	Manufacturing process	
Adventitious Agents	Bacteriophages	Unknown	Raw materials and manufacturing process	
	Bioburden (Contaminant)	Safety, Purity and Efficacy (degradation or modification of the product by contaminating microorganisms)	Bioburden can be introduced by raw materials and throughout the manufacturing process	
	Endotoxin (Contaminant)	Safety and Purity	Endotoxin can be introduced through raw materials and throughout the manufacturing process	
Composition and Strength	Protein Concentration	Bioactivity	Manufacturing process (b) (4)	
	Osmolality	Bioactivity	Formulation	
	pH	Bioactivity	Formulation	
	Appearance (clarity and color)	Safety	Product, formulation	
	(b) (4)			

DS - drug substance, DP - drug product, IPC – in process control

- **Description**: Caplacizumab-yhdp is a bivalent, single-domain immunoglobulin antibody construct produced in *E. coli* that consists of two identical humanized building blocks (denominated PMP12A2h1) that are genetically linked by a tri-alanine linker; it consists of 259 amino acids and has a molecular weight of 27876 Dalton.

- **Mechanism of Action (MoA)**: Caplacizumab-yhdp specifically binds to the A1 domain of vWF, inhibiting the interaction between vWF and platelets and thereby preventing vWF-mediated platelet aggregation.
- **Potency Assay**: (b) (4)
- **Reference Materials**: (b) (4)
- (b) (4)
-

(b) (4)

- **Container closure**: (b) (4)
- **Dating period and storage conditions**: (b) (4)

C. Drug Product, Cablivi, Quality Summary:

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes.

Table 3: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA type	CQA	Risk	Origin	Control Strategy
Appearance	Appearance of the cake	Immunogenicity, Safety	Lyophilization process, product, formulation	(b) (4)
	Appearance of the reconstituted solution: Clarity	Safety, PK/PD, immunogenicity	Product, formulation	

CQA type	CQA	Risk	Origin	Control Strategy
	Appearance of the reconstituted solution: Visible Particles	Safety, PK/PD, immunogenicity	DP manufacturing process, CCS, and product	
	Appearance of the reconstituted solution: Color	Safety, Immunogenicity	Product, formulation	
Quantity	Protein content	Safety, Biological activity, PK/PD, immunogenicity	Formulation	(b) (4)
	Uniformity of dosage units	Safety, Biological activity, PK/PD, immunogenicity	DP manufacturing (b) (4)	
	Protein concentration of the reconstituted solution	Safety, Biological activity, PK/PD, immunogenicity	Formulation	
Bioactivity	Potency of reconstituted solution	PK/PD, Biological activity, safety	Intrinsic to the molecule	
Contamination	Sterility	Safety, Purity, and Efficacy (degradation or modification of the product by contaminating microorganisms)	Contamination may be introduced throughout the DP manufacturing process or by container closure integrity failure	
	Endotoxin	Purity and Safety	DP manufacturing process and through raw materials	
	Container closure integrity	Safety (Failure in closure integrity may lead to contamination through a loss of sterility) or evaporation/leakage (impacting concentration or content)	May be impacted by storage conditions	
General	Moisture content	Safety, Biological activity, PK/PD, immunogenicity	DP lyophilization process.	
	pH	PK/PD, Biological activity, immunogenicity, safety	Formulation	

CQA type	CQA	Risk	Origin	Control Strategy
	Osmolality	PK/PD, Safety	Formulation	
	Reconstitution time	Safety, Biological activity, immunogenicity	Formulation	
	Appearance of the reconstituted solution: Sub-visible particles	PK/PD, safety, Immunogenicity	DP manufacturing process, CCS, and product	
Solvent (pre-filled syringe with water for injection)				
Content	Extractable volume	Biological activity, PK/PD	PFS manufacturing (b) (4)	(b) (4)
Appearance	Appearance of solution	Safety, immunogenicity	Product	
	Appearance of solution / Foreign insoluble matter	Safety, immunogenicity	DP manufacturing process, CCS, and product	
Contamination	Sterility	Safety, Purity, and Efficacy (degradation or modification of the product by contaminating microorganisms)	PFS manufacturing process, container closure integrity failure	
	Endotoxin	Purity and Safety	DP manufacturing process and through raw materials	
	Container closure integrity	Safety (Failure in closure integrity may lead to contamination through a loss of sterility)	May be impacted by storage conditions	
General	Particulate matter (subvisible)	PK/PD, safety, Immunogenicity	PFS manufacturing process, CCS, and product	
Functionality	Break loose force	Proper device functionality	Container closure components	
	Glide force			

- **Potency and Strength:** (b) (4) (11 mg nominal dose[7] after reconstitution in water using provided pre-filled syringe).
- **Summary of Product Design:** The DP is filled in 2R glass vials as sterile, lyophilized powder for solution for injection. (b) (4)

[7] The applicant proposes that the nominal dose after reconstitution is 10 mg. However, based on data provided by the applicant OPQ recommends that the product be labeled with the actual dose of 11 mg (refer to section E of this memo).

(b) (4)

The WFI used for reconstitution is provided in a pre-filled syringe with the drug product vial. Other accompanying components of the kit include: a vial adapter, 30 G hypodermic needle, and alcohol swabs.

- **List of Excipients:** Sucrose (b) (4) mg), polysorbate-80 (b) (4) mg), citric acid anhydrous (b) (4) mg), and trisodium citrate dihydrate (b) (4) mg), pH 6.5.
- **Reference Materials:** Same as caplacizumab-yhdp drug substance reference standards.
- **Manufacturing process summary for lyophilized product:**

(b) (4)

  - **Container closure:** 2R Type (b) (4) glass vial stoppered with a (b) (4) rubber stopper. The stoppers are capped with an aluminum crimp seal equipped with a (b) (4) tamper-evident flip-off cap.
  - **Dating period and storage conditions:** 48 months at 2°C – 8 °C.

- **Manufacturing process summary for WFI pre-filled syringe (solvent):**

(b) (4)

(b) (4)

- **Container closure for PFS:** Syringe barrel (b) (4) glass, (b) (4) and a rubber stopper (b) (4)
- **Dating period and storage conditions for PFS:** (b) (4)

The expiration date of each kit is determined by the expiration date of the component expiring first and will be printed in MMM YYYY format on the secondary packaging, the vial, and the syringe label.

D. Novel Approaches/Precedents: None

E. Any Special Product Quality Labeling Recommendations:

1. In the original submission and labeling materials, the applicant states that:
   - the strength of the product is 10 mg of caplacizumab-yhdp as lyophilized, sterile powder
   - (b) (4)

   However, the proposed commercial control strategy results in a product that consistently delivers 11 mg of caplacizumab-yhdp. Data provided as part of the human factor studies (74036D009 and 74036D020), vial adapter usability studies (GMP-MEMO-0041-v01), the dose recovery study (GMP-R-0228-v01), and in response to the Agency's information request dated October 13, 2018 (GMP-R-0370-v01), supports the conclusion that the actual dose of the caplacizumab-yhdp is **11 mg** and the deliverable volume is **1 mL** when the instructions for use (IFU) are followed (same instructions used during the pivotal clinical trial ALX-0681-C-301). Per the IFU the product is reconstituted with the provided solvent PFS "until the syringe is empty" and then it is withdrawn by "slowly pull the plunger to withdraw all of the solution from the vial into the syringe." The syringe is then detached from the vial and it is used to administer the dose.

   Recommendation:
   OPQ recommends that the labeling materials be updated to reflect the actual extractable amount of reconstituted product (dose) of 11 mg (11 mg/mL).

   This was communicated and discussed with the applicant during the late cycle meeting.

2. Section 6.2 Immunogenicity of the package insert (b) (4) The product quality review team consulted with the OBP Immunogenicity Working Group (IWG) regarding the acceptability of the Applicant's

strategy for detection of anti-drug antibodies (ADA) and treatment-emergent ADA (TE-ADA) and the proposed label.

Recommendation:
OBP recommends that the Immunogenicity section of the label includes a discussion around the presence of baseline positive samples (i.e., pre-existing antibodies as referred to by the applicant) which are present in 55 of 97 subjects in the caplacizumab-yhdp treatment group and 46 of 73 subjects in the placebo arm. (b) (4)
It is current practice to include this information on the PI and it is in line with other labels for biotechnology products for which pre-existing antibodies have been detected. This was discussed with the applicant during the late cycle meeting and they agreed to propose updated immunogenicity language to include this information.

F. Establishment Information:

Overall Recommendation: **Approval**					
DRUG SUBSTANCE					
Function	Site Information	FEI/DUNS	Preliminary Assessment	Inspectional Observations	Final Recommendation
(b) (4)			NAI	(b) (4) No Form 483 was issued.	Approval
			NAI	Last inspection was completed (b) (4) and was a surveillance inspection. No Form 483 was issued.	Approval
			NAI	The last establishment inspection was a (b) (4) A Form FDA 483, was issued for the following: (b) (4)	Approval

				(b) (4)	
-QC testing for batch release and stability testing[1]	Ablynx NV Technologiepark 21 9052 Zwijnaarde Belgium	DUNS No. 372000096 FEI No. 3008915297	NAI	A PLI was conducted from (b) (4) No Form 483 was issued.	Approval

[1]Appearance (clarity, color), Protein content by (b) (4)

DRUG PRODUCT					
Function	Site Information	DUNS/FEI Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
(b) (4)			NAI	A PLI was conducted from (b) (4) No Form 483 was issued.	Approval
- Batch release - QC testing for batch release and stability testing[1] - QC testing for stability testing (Moisture content, Container closure integrity)	Ablynx NV Technologiepark 21 9052 Zwijnaarde Belgium	DUNS No. 372000096 FEI No. 3008915297	NAI	A PLI was conducted from 09/24/2018 to 09/26/2018.No Form 483 was issued.	Approval
(b) (4)			NAI	Last inspection was completed (b) (4) and was a surveillance inspection. No Form 483 was issued.	Approval

[1]Appearance of the cake, Appearance of the reconstituted solution (clarity, color, visible particles), Protein content by (b) (4) (test not performed in stability), Uniformity of dosage units (test not performed in stability), Protein concentration of the reconstituted solution by (b) (4) (test not performed for release), (b) (4) Reconstitution time.

WFI Pre-Filled Syringe (Solvent)					
Function	Site Information	FEI/DUNS Number	Preliminary Assessment	Inspectional Observations	Final Recommendation
(b) (4)			VAI	Last inspection was completed (b) (4) and was	Approval

(b) (4)		a surveillance inspection. (see below for more info) No PLI was requested.	
	VAI	Last inspection was completed (b) (4) and was a pre-approval inspection. No PLI was requested.	Approval
	VAI	Last inspection was completed (b) (4) and was a surveillance inspection. No PLI was requested.	Approval
	VAI	Last inspection was a surveillance inspection conducted (b) (4) No PLI was requested.	Approval

G. Facilities:

- (b) (4)
-
-

Final facility recommendation: Acceptable/ Approval

- (b) (4)

Final facility recommendation: Acceptable/ Approval

- For the status of all other facilities included in the application refer to Section F: Establishment Information of this document.

H. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols submitted to the BLA:

1. (b) (4)
2.
3. Post-Approval Stability Program (3.2.S.7.2)

ii. Outstanding review issues/residual risk:

See Post Marketing Commitments in section I. D.

iii. Future inspection points to consider: None identified.

b. Drug Product

i. Protocols submitted to the BLA:

1. (b) (4)
2. Post-Approval Stability Program (3.2.P.8.2)

ii. Outstanding review issues/residual risk:

See Post Marketing Commitments in section I. D.

iii. Future inspection points to consider: None identified.

c. Summary of commitments from the Applicant

4 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page

Quality Assessment Summary Tables

Table 1: Noteworthy Elements of the Application

#	Checklist		Yes	No	N/A
Product Type					
1.	Recombinant Product		X		
2.	Naturally Derived Product			X	
3.	Botanical			X	
4.	Human Cell Substrate/source material			X	
5.	Non-Human Primate Cell Substrate/Source Material			X	
6.	Non-Primate Mammalian Cell Substrate/source material			X	
7.	Non-Mammalian Cell Substrate/Source Material		X		
8.	Transgenic Animal source			X	
9.	Transgenic Plant source			X	
10.	New Molecular Entity		X		
11.	PEPFAR drug			X	
12.	PET drug			X	
13.	Sterile Drug Product		X		
14.	Other: First-in-class		X		
REGULATORY CONSIDERATIONS					
15.	Citizen Petition and/or Controlled Correspondence Linked to the Application [fill in number]			X	
16.	Comparability Protocol(s)			X	
17.	End of Phase II/Pre-BLA Agreements		X		
18.	SPOTS (special products on-line tracking system)			X	
19.	USAN assigned name		X		
20.	Other [Fast-track designation with priority review]		X		
QUALITY CONSIDERATIONS					
21.	Drug Substance Overage			X	
22.	Design Space	Formulation		X	
23.		Process		X	
24.		Analytical Methods		X	
25.		Other		X	
26.	Other QbD Elements		X		
27.	Real Time release testing (RTRT)			X	
28.	Parametric release in lieu of Sterility testing			X	
29.	Alternative Microbiological test methods			X	
30.	Process Analytical Technology in Commercial Production			X	
31.	Non-compendial analytical procedures	Drug Product	X		
32.		Excipients		X	
33.		Drug Substance	X		
34.	Excipients	Human or Animal Origin		X	
35.		Novel		X	
36.	Nanomaterials			X	
37.	Genotoxic Impurities or Structural Alerts			X	
38.	Continuous Manufacturing			X	
39.	Use of Models for Release			X	
40.	Other				X

Review documents related to this Executive Summary (links to document in Panorama)**:**

- Combined drug substance and drug product quality assessment by Jacek Cieslak, PhD, and human immunogenicity assays assessment by Leopold Kong, PhD (OPQ/OBP/DBRR IV)
- Drug substance microbiology assessment by Scott Nichols, PhD (OPQ/OPF/DMA IV)
- Drug product microbiology assessment by Candace Gomez-Broughton, PhD (OPQ/OPF/DMA IV)
- Facility assessment by Viviana Matta (OPQ/OPF/DIA)
- Device evaluation assessment by Florencia Wilson, RN,BSN,CCRN (CDRH/ODE)
- OBP labeling assessment by Vicky Borders-Hemphill, PharmD (OPQ/OBP)

Leslie Rivera-Rosado

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Christopher Downey

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Reyes Candau-Chacon

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Zhihao Peter Qiu

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DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Drug Evaluation and Research
WO Bldg. 51, 10903 New Hampshire Ave.
Silver Spring, MD 20993

Date: 12/18/18
To: Administrative File, STN 761112/0
From: Viviana Matta, Consumer Safety Officer, CDER/OPQ/OPF/DIA
Endorsement: Peter Qiu, Ph.D., Branch Chief, CDER/OPQ/OPF/DIA
Subject: New Biologic License Application (BLA)
US License: 2085
Applicant: Ablynx NV
Mfg Facility: Drug Substance: (b) (4)
(b) (4)
Drug Product: (b) (4)
Product: Cablivi (caplacizurnab)
Dosage: (b) (4) / Pre-filled syringe
Indication: (b) (4)
Due Date: 02/06/19

Recommendation: The proposed manufacturing and testing sites are recommended for approval from a facilities assessment standpoint.

SUMMARY

The subject BLA proposes manufacture of ALX-0081 Drug Substance and Cablivi Drug Product at the following facilities.

(b) (4)

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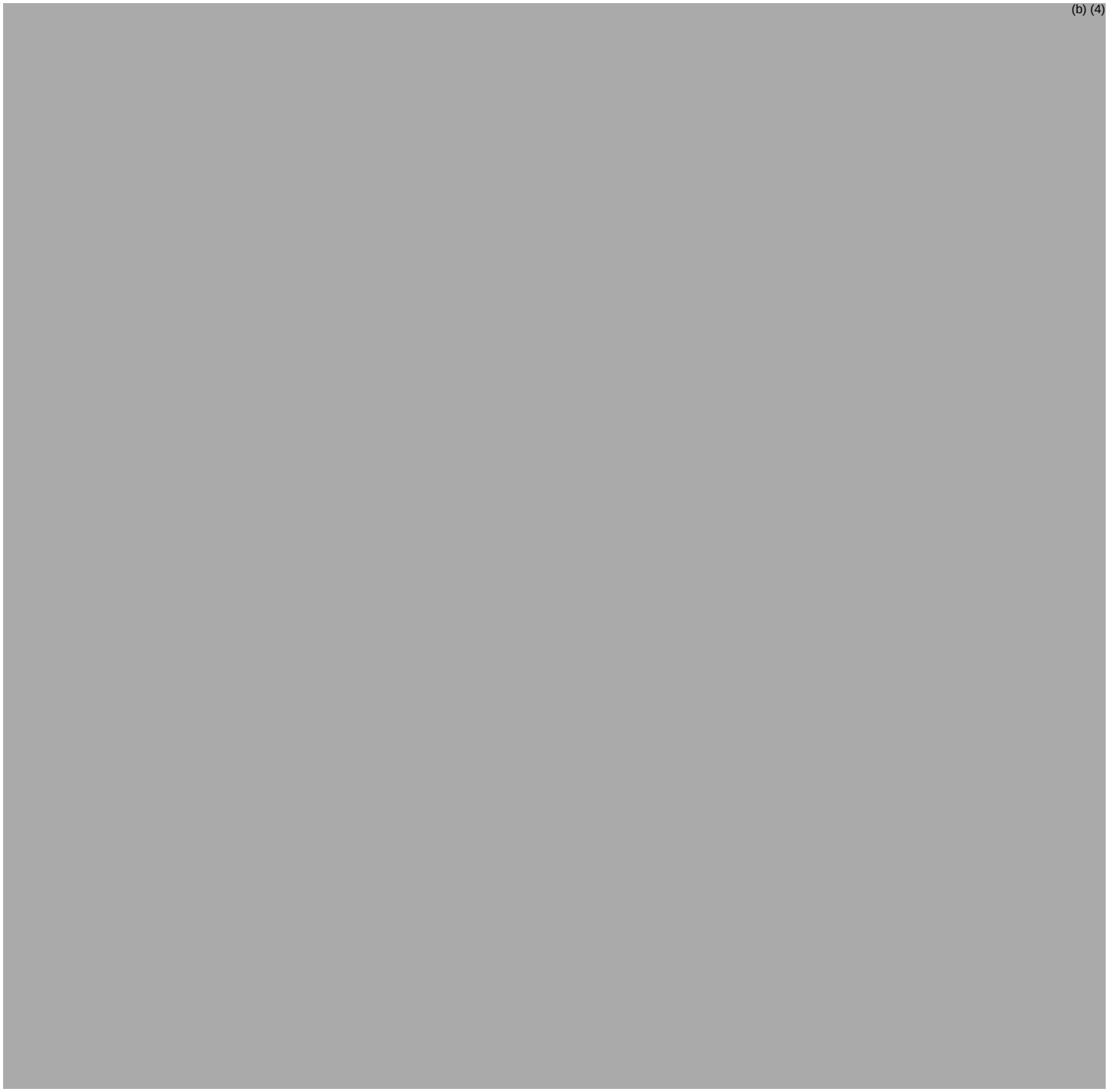
(b) (4)



The proposed manufacturing and testing site are recommended for approval from a facilities assessment standpoint.

Viviana Matta -S

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Viviana Matta
Consumer Safety Officer
OPF Division of Inspectional Assessment
Branch 1

Zhihao Qiu -S

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Zhihao (Peter) Qiu, Ph.D.
Branch Chief
OPF Division of Inspectional Assessment
Branch 1

Viviana Matta

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Zhihao Peter Qiu

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U.S. FOOD & DRUG ADMINISTRATION

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment

Date: November 20, 2018
STN: 761112/0
Reviewer: Candace Gomez-Broughton, Ph.D. Microbiologist CDER/OPQ/OPF/DMA/Branch IV
Endorsed: Reyes Candau-Chacon, Ph.D., Quality Assessment Lead CDER/OPQ/OPF/DMA/Branch IV
Subject: Original Biologics License Application (BLA)
Applicant: Ablynx NV
Facilities: (b) (4)
Product: CABLIVI® (caplacizumab, ALX-0081)
Dosage: powder and solvent for solution for IV and SC injection
Indication: (b) (4)
Action Date: February 6, 2019

Recommendation: This BLA is recommended for approval from a microbiology product quality perspective with the following post-marketing commitments:

1. To conduct a study to demonstrate that the pre-filled syringe plunger movement during transport does not impact product sterility.
2. To repeat the (b) (4) bacterial retention study using a non-bactericidal surrogate solution with physical attributes comparable to the product.

Introduction

Ablynx has submitted a biological license application BLA for the approval of CABLIVI® (caplacizumab, ALX-0081) for treatment of patients with acquired thrombotic purpura (aTTP). The ALX-0081 drug product (DP)is lyophilized and packaged with syringes filled with sterile water for injection (sWFI) for reconstitution.

This review covers the manufacturing processes for the drug product and sWFI syringes. The drug substance process is covered in a separate review completed by Scott Nichols, Ph.D.

Amendments Reviewed

- Sequence 0014 submitted 05 Oct 2018
- Sequence 0022 submitted 16 Nov 2018
- Sequence 0025 submitted 27 Nov 2018

Assessment

1.14 Labeling

The lyophilized product is stored at 2-8°C or at room temperature for up to two months. The reconstituted solution can be kept for up to 4 hours at 2-8°C.

Reviewer comment: Labeling instructions for storage are adequate.

P. Drug Product

P. Cablivi – Powder - (b) (4)

P.1 Description and Composition of the Drug Product

DP is filled in 2R glass vial as a sterile, lyophilized powder. The DP is reconstituted with 1 mL WFI provided in co-packaged pre-filled syringes (PFS) to allow for a 10 mg dose. The DP formulation consists of ALX-0081, citric acid anhydrous, trisodium citrate dehydrate, sucrose, polysorbate 80, and WFI.

The DP container closure system consists of 2R glass vials (type (b) (4) glass), (b) (4) rubber stopper, and aluminum crimp seal. The final DP is included in a kit. The accompanying components are listed below.

- Solvent – 1 mL sterile WFI for reconstitution (described in Section 3.2.P Solvent)
- Vial adapter provided for reconstitution (described in Section 3.2.R)
- Needle – single (b) (4) Hypodermic 30G Safety Needle (described in Section 3.2.R) for administration of reconstituted DP
- Two alcohol skin wipes for disinfection of the injection site and for cleaning the rubber stopper of the DP vial

<u>Reviewer comment</u>: The DP has been adequately described. Components other than DP vial and solvent syringe are reviewed by The Center for Devices and Radiological Health (CDRH).

P.2 Pharmaceutical Development

(b) (4)

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Reyes Candau-Chacon

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BLA STN 761112

Cablivi (caplacizumab-yhdp)
Ablynx NV

Jacek Cieslak, Ph.D., Chemist
Leopold Kong, Ph.D., Chemist
Leslie A. Rivera Rosado, Ph.D., Team Lead
Christopher Downey, Ph.D., Review Chief

Office of Biotechnology Products
Division of Biotechnology Review and Research IV

OBP CMC Review Data Sheet

1. **BLA#:** STN 761112 \\CDSESUB1\evsprod\BLA761112\761112.enx

2. **REVIEW DATE:** December 7, 2018

3. **PRIMARY REVIEW TEAM:**

Product Quality Team:

Discipline	Reviewer	Center/ Office/Division
Drug Substance (DS)	Jacek Cieslak	CDER/OPQ/OBP/DBRR IV
Drug Product (DP)	Jacek Cieslak	CDER/OPQ/OBP/DBRR IV
Immunogenicity Assays	Leopold Kong	CDER/OPQ/OBP/DBRR IV
Labeling	CAPT Vicky Borders Hemphill	CDER/OPQ/OBP
Facilities	Viviana Matta	CDER/OPQ/OPF/DIA
Microbiology	Scott Nichols (DS) Candace Gomez-Broughton (DP)	CDER/OPQ/OPF/DMA IV
Device	Florencia Wilson	CDRH/ODE/DAGRID/GHDB
Team Leads	LCDR Leslie A. Rivera Rosado (Product Quality) Peter Qiu (Facilities) Reyes Candau-Chacon (Microbiology)	CDER/OPQ/OBP/DBRR IV CDER/OPQ/OPF/DIA CDER/OPQ/OPF/DMA IV
OPQ RBPM	Melinda Bauerlien	CDER/OPQ/OPRO
OPQ Application Team Lead	LCDR Leslie A. Rivera Rosado	CDER/OPQ/OBP/DBRR IV

Multidisciplinary Review Team:

Discipline	Reviewer	Center/Office/Division
RPM	Beatrice Kallungal and Laura Wall	CDER/OND/OHOP/DHP
Cross-disciplinary Team Lead	Kathy Robie Suh	CDER/OND/OHOP/DHP
Medical Officer	Andrew Dmytrijuk, Heather Fitter (TL)	CDER/OND/OHOP/DHP
Clinical Safety	Virginia Kwitkowski	CDER/OND/OHOP/DHP
Pharm/Tox	Brenda Gehrke, Christopher Sheth (TL)	CDER/OND/OHOP/DHOT
Clinical Pharmacology	Robert Schuck, Olanrewaju Okusanya	CDER/OTS/OCP/DCPV
Pharmacometrics/PBPK	Justin C Earp, Junshan Qiu	CDER/OTS/OCP/DPM
Statistics	Kunthel By, Yuan Li Shen	CDER/OTS/OB/DBV
Pharmacovigilance	Regina Lee, Peter Waldron	CDER/OSE/OPE/DPVI I
Epidemiology	Carolyn McCloskey	CDER/OSE/OPE/DEPI I
Medication Error Prevention and Analysis	Nicole Garrison Hina Mehta	CDER/OSE/OMEPRM/DMEPA
Risk Management	Naomi Redd, Elizabeth Everhart	CDER/OSE/OMEPRM/DRISK

4. **MAJOR GRMP DEADLINES**
Filing Meeting: July 5, 2018
Mid-Cycle Meeting: September 3, 2018 (OPQ) and September 4, 2018 (OND)
Primary Review Due: December 14, 2018
Wrap-Up Meeting: December 17, 2018
PDUFA Action Date: February 6, 2019

5. **COMMUNICATIONS WITH APPLICANT:**

Communication/Document	Date
Information Request #1 (OPF)	April 17, 2018
Information Request #2 (OPF)	July 10, 2018
Information Request #3 (OBP Labeling)	August 2, 2018
Information Request #4 (OPF/DMA)	August 7, 2018
Information Request #5 (OBP, CDRH)	September 14, 2018
Mid-Cycle Communication (Teleconference)	September 17, 2018
Information Request #6 (OPF/DMA)	September 20, 2018
Mid-Cycle Communication (background package)	September 26, 2018
Information Request #7 (OPF/DMA)	October 11, 2018
Information Request #8 (OBP)	October 13, 2018
Information Request #9 (OBP)	October 31, 2018
Information Request #10 (OPF/DMA)	November 5, 2018
Information Request #11 (OBP Immuno assays)	November 8, 2018
Information Request #12 (OBP)	November 16, 2018
Information Request #13 (OPF/DMA, CDRH)	November 21, 2018
Late-Cycle Communication (background package)	November 23, 2018
Information Requeste #14 (OBP)	December 5, 2018
Late-Cycle Meeting with the applicant (F-to-F)	December 7, 2018

6. **SUBMISSION(S) REVIEWED:**

Submission	Date Received	Review Completed by:
STN 761112/1- BLA Original Application	4/4/2018	All
STN 761112/2- Response to 4/17/2018 IR	4/25/2018	OPF
STN 761112/3- Update to response to 4/17/2018 IR	4/27/2018	OPF
STN 761112/4- Rolling submission part 2	6/6/2018	All
STN 761112/7- Response to 7/10/2018 IR	7/13/2018	OPF
STN 761112/10- Labeling/PI draft	8/2/2018	OBP Labeling
STN 761112/11- Response to 8/7/2018 IR	8/22/2018	OPF/DMA
STN 761112/12- Response to 9/14/2018 IR	9/24/2018	OBP, CDRH device
STN 761112/15- Response to 9/20/2018 IR	10/5/2018	OPF/DMA
STN 761112/17- Response to 10/11/2018 IR	10/19/2018	OPF/DMA
STN 761112/18- Response to 9/20/2018 IR	10/23/2018	OPF/DMA
STN 761112/19- Response to 8/7/2018 IR	11/2/2018	OPF/DMA
STN 761112/20- Response to 10/13/2018 IR	11/2/2018	OBP
STN 761112/21- Response to 10/31/2018 IR	11/13/2018	OBP
STN 761112/22- Response to 11/8/2018 IR	11/14/2018	OBP (Immuno assays)
STN 761112/23- Response to 10/31/2018 & 11/5/2018 IRs	11/16/2018	OPF/DMA
STN 761112/24- Response to 11/16/2018 IR	11/21/2018	OBP
STN 761112/25- Response to 10/31/2018 IR	11/26/2018	CDRH
STN 761112/26- Response to 11/21/2018 IR	11/27/2018	OPF/DMA, CDRH
STN 761112/26- Response to 10/31/2018, 11/5/2018, 11/16/1028 & 11/21/2018 IRs	11/30/2018	OBP, OPF/DMA
STN 761112/27- Response to 12/5/2018 IR	12/10/2018	All

7. **DRUG PRODUCT NAME/CODE/TYPE:**
   a. Proprietary/trade Name: Cablivi (cab-LIV-ee)
   b. Non-Proprietary/USAN: caplacizumab-yhdp
   c. CAS name: 915810-67-2
   d. Common name: ALX-0081
   e. INN Name: caplacizumab
   f. Compendial Name: N/A
   g. OBP systematic name[1]: MABFRAG HUMANIZED BIVALENT ANTI P04275 (VWF_HUMAN) [ALX0081]
   h. Other Names: N/A

8. **PHARMACOLOGICAL CATEGORY:** von Willebrand factor-directed antibody construct (pending)

9. **DOSAGE FORM:** Injection

10. **STRENGTH/POTENCY:** (b) (4) (10 mg nominal dose[2] after reconstitution in water using provided pre-filled syringe).

11. **ROUTE OF ADMINISTRATION:** Intravenous and Subcutaneous

12. **REFERENCED MASTER FILES:**

DMF #	DMF type	HOLDER	ITEM REFERENCED	Letter of Cross-Reference	COMMENTS (STATUS)
(b) (4)	III	(b) (4)		Yes	Sufficient information was provided in the BLA for its intended use.
	III			Yes	Sufficient information was provided in the BLA for its intended use.
	III			Yes	Sufficient information was provided in the BLA for its intended use.

[1] The OBP systematic name allows searching for related products in OBP's database and in the Document Archiving, Reporting & Regulatory Tracking System (DARRTS) for safety reasons and it is different from the non-proprietary name. The tag at the end is used to separate products from different applicants and it is generally the name used by applicants to refer to the proposed product in their submissions.

[2] The applicant proposes that the nominal dose after reconstitution is 10 mg. However, based on data provided by the applicant OPQ recommends that the product is labeled with the actual dose of 11 mg (refer to section 3.2.P.1 Description and Composition of the Drug Product of this document)

13. INSPECTIONAL ACTIVITIES

- A pre-license inspection of the caplacizumab drug substance manufacturing facility at [redacted] was conducted on [redacted] by OPQ/OPF/DMA and OPQ/OBP/DBRR IV in support of this BLA. No FDA 483 form was issued to the firm and a final facility recommendation was NAI.

- A pre-license inspection of Cablivi drug product manufacturing facility at [redacted] was conducted on [redacted] by OPQ/OPF/DIA in support of this BLA. No FDA 483 form was issued to the firm and a final facility recommendation was NAI.

- A pre-license inspection of quality control testing site at Ablynx N.V. Technologiepark 21 9052 Zwijnaarde Belgium (FEI 3008915297) was conducted on September 24 – 26, 2018 by ORA in support of this BLA. No FDA 483 form was issued to the firm and a final facility recommendation was NAI.

14. CONSULTS REQUESTED BY OBP

Discipline/Topic	Date Requested	Status	Recommendation	Reviewer
CDRH/technical/engineering Review of delivery device	8/13/2018	Completed 12/06/2018	The device constituent of the combination product is **approvable** for the proposed indication.	CDRH-ODE: Florencia Wilson, Carolyn Dorgan (TL)
CDRH/Facilities inspections	8/13/2018	Completed 12/06/2018	CDRH does not need to conduct a compliance evaluation of the application.	CDRH-OC: M. Isabel Tejero del Rio

15. QUALITY BY DESIGN ELEMENTS

The following was submitted in the identification of QbD elements (check all that apply):

	Design Space
x	Design of Experiments
x	Formal Risk Assessment / Risk Management
	Multivariate Statistical Process Control
	Process Analytical Technology
	Expanded Change Protocol

Risk assessments to identify critical quality attributes of caplacizumab and to identify process parameters for assessment in process characterization studies were performed according to methods described in the submission and review of Module 3.
A design of experiments (DoE) approach was utilized to generate process understanding. Results from DoE experiments were used to support the overall control strategy proposed for caplacizumab drug substance and drug product. The applicant does not claim a design space.

16. PRECEDENTS

None

17. ADMINISTRATIVE

A. Signature Block

Name and Title	Signature and Date
Christopher Downey, Ph.D. Review Chief Division of Biotechnology Review and Research IV (DBRR IV) Office of Biotechnology Products (OBP) Office of Pharmaceutical Quality (OPQ)	See attached
LCDR Leslie Ann Rivera Rosado Product Quality Team Leader DBRR IV, OBP, OPQ	See attached
Jacek Cieslak, Ph.D. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached
Leopold Kong, Ph.D. Product Quality Reviewer DBRR IV, OBP, OPQ	See attached

B. CC Block

Recipient	Date
Beatrice Kallungal Clinical Division BLA RPM	
Laura Wall Clinical Division BLA RPM	
OBP/DBRR IV File/BLA STN 761112	

SUMMARY OF QUALITY ASSESSMENTS

I. Primary Reviewer Summary Recommendation

The Office of Biotechnology Products (OBP) recommends approval of BLA 761112 for Cablivi (caplacizumab) manufactured by Ablynx. The data submitted in this Biologics License Application adequately support the conclusion that the manufacture of Cablivi is well controlled and leads to a product that is pure and potent. The conditions used in manufacturing have been adequately validated by manufacture of multiple drug substance and drug product batches.

The proposed shelf life of formulated drug substance is (b) (4) months at (b) (4) °C. The proposed shelf life for drug product is 48 months at 5 ± 3 °C. The proposed shelf lives are supported by the stability data and are recommended for approval. OBP recommends approval of the proposed lot release and stability specifications and stability protocols for caplacizumab drug substance and drug product. It is recommended that Cablivi be approved for human use and under conditions specified in the package insert.

II. List Of Deficiencies To Be Communicated
Not applicable

III. List Of Post-Marketing Commitments/Requirement

[Draft Language]
To develop and implement an analytical method to determine polysorbate 80 (PS80) levels as an (b) (4) control on ALX-0081 drug product (DP) with appropriate upper and lower (b) (4) The final report, including method validation reports and assessment of acceptance criteria for polysorbate 80 based on method capabilities and available data, should be submitted as a Prior Approval Supplement.

IV. Review Of Common Technical Document-Quality Module 1

In Module 1 (1.12.14 Environmental Assessment – Claim for Categorical Exclusion), Ablynx NV is requesting a categorical exclusion under 21 CFR 25.31(a) from the need to prepare an environmental assessment based on the calculations of the expected introduction concentration of an active moiety.

Reviewer comment: *The claim of categorical exclusion is accepted.*

V. Primary Container Labeling Review
Refer to review by CAPT Vicky Borders-Hemphill.

VI. Review Of Common Technical Document- Quality Module 3.2
This document.

VII. Review Of Immunogenicity Assays – Module 5.3.1.4
This document.

Table of Content

3.2.S. DRUG SUBSTANCE

3.2.S.1 General Information

Caplacizumab is a bivalent, single-domain immunoglobulin antibody construct, termed a "Nanobody[3]" by the applicant, produced in *E. coli* by standard recombinant expression technology, which is directed towards the human A1 domain of von Willebrand factor (vWF). It binds to the A1 domain of vWF and specifically inhibits the interaction between vWF and platelets. Caplacizumab can interact with vWF in both its active (*i.e.* ultra-large (UL)vWF multimers or normal multimers activated through immobilization or shear stress) and inactive forms (*i.e.* multimers prior to conformational change of A1 domain), thereby immediately blocking the interaction of vWF with the GPIb-IX-V platelet receptor. As such, it prevents spontaneous interaction of ULvWF with platelets that would otherwise lead to platelet microthrombi formation in the microvasculature, local schema, and platelet consumption.

Caplacizumab is indicated for treatment of adults with acquired thrombotic thrombocytopenic purpura (aTTP) through inhibition of the micro clot formation responsible for the significant mortality and morbidity associated with this disease.

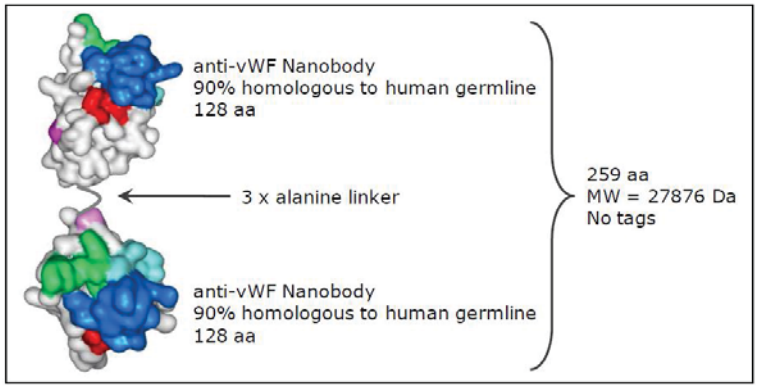
3.2.S.1.1 Nomenclature

Descriptive Names: ALX-0081 bivalent, single-domain immunoglobulin antibody construct directed towards the human Al domain of von Willebrand factor
International Non-proprietary Name (INN): caplacizumab
United States Adopted Name (USAN): caplacizumab-yhdp
Chemical Abstracts Service (CAS) Registry Number: 915810-67-2
Company Name: ALX-0081

3.2.S.1.2 Structure

ALX-0081 is a bivalent antibody construct produced in *E. coli* that consists of two identical humanized building blocks that are genetically linked by a tri-alanine linker. ALX-0081 consists of 259 amino acids and has a molecular weight of 27876 Dalton. A schematic representation is shown in Figure 1 below (copied from the submission).

Figure 1: Schematic structure of ALX-0081



[3] "Nanobody" is a registered trademark of Ablynx N.V.

CDR (complementary determining region)-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 (camelid variable heavy chain region) compared to a human VH (variable heavy chain region).

The caplacizumab amino acid sequence is provided in Figure 2 in Section 3.2.S.1.2 with the CDR highlighted.

3.2.S.1.3 General Properties

Caplacizumab general properties are summarized in Table 1.

Table 1: General properties of ALX-0081 drug substance

Appearance	Clear liquid, which is colorless or slightly brown
Molecular weight	27876 Da
Isoelectric point	(b) (4)
Extinction coefficient ε	1.50 $mL.mg^{-1}.cm^{-1}$
Protein concentration	(b) (4) mg/mL
pH (room temperature)	
Osmolality	mOsm/kg
Biological activity	Biacore potency assay (b) (4) U/mg

3.2.S.2 Manufacture

(b) (4)

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5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Assessment of human Immunogenicity Assays
by Leopold Kong, Ph.D., Product Quality Reviewer CDER/OPQ/OBP/DBRR IV

Summary

Caplacizumab (ALX-0081) is a humanized recombinant antibody construct (referred to as nanobody® variable domain dimer by the Applicant) directed towards the human A1 domain of von Willebrand Factor (vWF). The Applicant states that caplacizumab has low immunogenicity risk because it consists of two humanized antibody domains and because the patients undergo co-treatment with immunosuppressants. To evaluate the immunogenicity of caplacizumab, the Applicant developed three anti-drug antibody (ADA) binding assay sets and two neutralizing antibody (NAb) assay sets. The three ADA binding assays sets include two Meso Scale Discovery (MSD) electrochemiluminescence (ECL) based homogenous bridging assays used in testing phase 1, 2 and 3 samples and a sequential enzyme-linked immunosorbent assay (ELISA) based bridging assay used initially for phase 1 and 2 samples. The two NAb assay sets include a functional ELISA-based assay used in testing phase 2 and 3 samples and an alternative ECL based bridging assay used in testing phase 3 samples. Of the two MSD ECL-based ADA binding assay sets, one is a conventional bridging assay displaying sufficient sensitivity and drug tolerance. The second ECL-based ADA binding assay set was developed to specifically detect treatment-emergent ADA and to have reduced binding to pre-existing antibodies (pre-Ab), which was prevalent among the clinical study subjects. Of the two NAb assay sets, one is a functional assay directly measuring the ability of ADA in samples to inhibit caplacizumab from inhibiting vWF from binding to platelets. However, the functional assay is not drug tolerant, and can only be used to analyze pre-dose or wash-out samples. The second NAb assay set is a drug-tolerant MSD ECL-based ADA binding bridging assay that was developed to specifically detect neutralizing ADA and to have reduced binding to non-neutralizing ADA. Overall, the Applicant's approach to validate the immunogenicity assays are appropriate and the data presented support the use of the assays in analyzing clinical samples.

Recommendation

The Immunogenicity section of the label should include a discussion around the presence of baseline positive samples (i.e., pre-existing antibodies as referred to by the Applicant), which were found to be present in 55 of 97 subjects in the caplacizumab treatment group and 46 of 73 subjects in the placebo arm of the phase 3 HERCULES study. (b) (4) complete description of the immunogenicity profile of the product.

Information Requests and Amendments

An information request (IR) was sent on 11/8/2018 during the review of the immunogenicity assays. The Applicant responded on 11/14/2018 in eCTD 0021 (22).

Review

- Reviewer's comments throughout the document are highlighted in *blue italics*. Unless otherwise noted, figures and tables in the review were adapted or copied directly from the submission.
- The review sequence of the individual aspect of the assay validation may not follow the exact sequence in the submission.
- The "guidance" cited in the review refer to "FDA Draft Guidance to Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins, April 2016" http://www.fda.gov/downloads/Drugs/.../Guidances/UCM192750.pdf

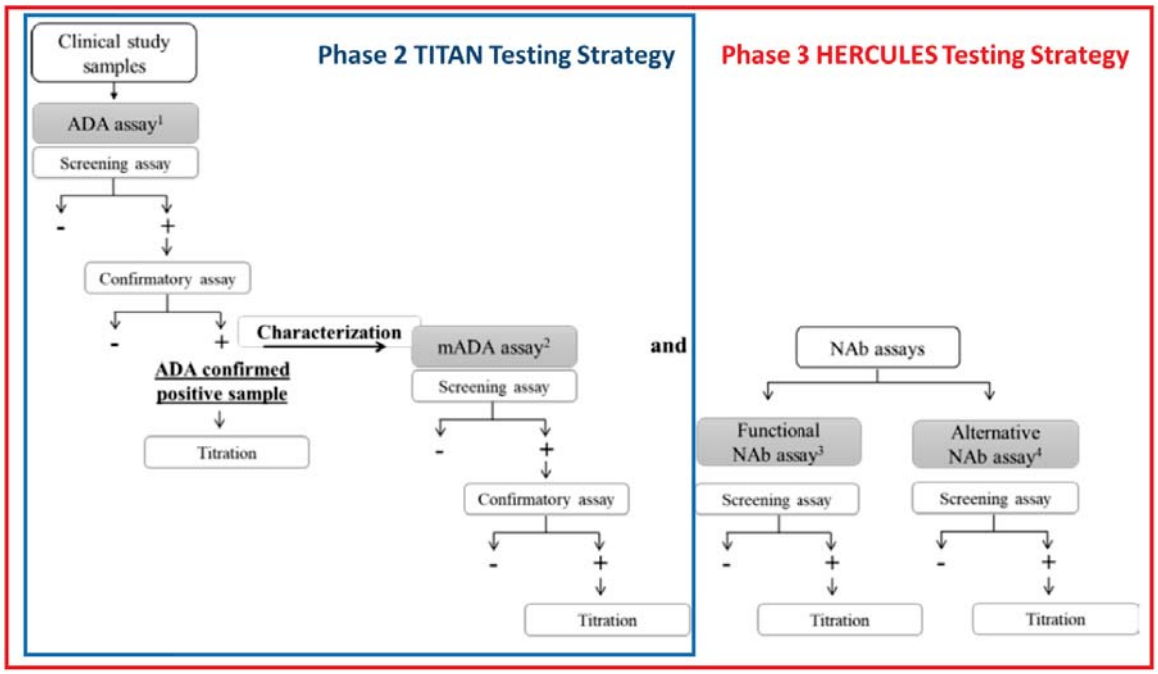
Background and Testing Strategy

The proposed treatment for patients with acquired thrombotic thrombocytopenic purpura (aTTP) begins upon diagnosis of aTTP. On the first day of treatment, patients receive an IV injection of the drug product (DP) prior to plasma exchange (PE) followed by a subcutaneous injection. Subsequent days of treatment include daily subcutaneous injections following plasma exchange. After the plasma exchange period, the patient receives subcutaneous injections daily for 30 days. Throughout treatment, the patients also receive concomitant treatments with immune suppressors such as Rituximab, which recognizes CD20 and depletes B cells, or more commonly, corticosteroids. During the phase 3 study ALX0681-C301(HERCULES), patient samples were collected for immunogenicity testing on day 1 during the daily PE period, and on days 1 and 30 during the 30-day post-daily PE period. Notably, all patients in the phase 3 study were treated with PE prior to the immunogenicity sampling, and consequently no ADA pre-dose dose was available to determine baseline pre-Ab status. Samples were also collected during first and final follow up visits (7 and 28 days after last dosing respectively). During the ongoing phase 3 study ALX0681-C301, samples are taken on screening day, recurrence visit, day 3 visit, week 1 visit (post daily PE day 1) and on weekly visits until the end of treatment as well as on every follow up visit.

PE confounds ADA results, as indicated by ADA positive samples in placebo groups after the PE exchange period.

The testing strategy to detect ADA evolved during the clinical development process. Initially, the Applicant detected ADA using a sequential bridging ELISA with low drug tolerance. Only one study (ALX-0081-2.1/09) using this approach conducted further tests on positive samples, which were titered, and further characterized using a functional neutralizing antibody (NAb) assay.

As clinical development proceeded, the Applicant observed a high incidence of pre-Ab in subjects either from the subject prior to treatment or from the PE. Therefore, beginning with the phase 2 clinical study ALX-0681-2.1/10 (TITAN), the Applicant adopted a dual assay approach using two drug-tolerant ECL-based assay sets as summarized within the **blue box** of the schematic below (schematic from Figure 1B of *Section 1.11.3 SN0021 RfI Clinical Pharmacology* of the submission in response to the IR sent 11/8/2018):



Briefly, the clinical samples were first tested in an ECL based conventional ADA screening assay. Samples that screened positive were tested in a confirmatory assay under the same format. Confirmed positive samples were then titered under the same format as well as further tested using the second ECL-based assay set that the Applicant proposes has a higher specificity for treatment emergent (TE) ADA (mADA assay; see below for how this specificity is achieved). No NAb assays were performed. In the phase 3 HERCULES study, confirmed positive samples were tested for neutralizing activity using either a functional NAb assay with low drug tolerance if the sample was pre-dose or washout, or an alternative drug-tolerant NAb assay that is in the same format as the ECL based ADA binding assays **(red box** in the schematic above). The results of the ADA binding assays for both the Phase 2 TITAN and Phase 3 HERCULES studies are used to classify the ADA response as summarized below:

1.) Positive conventional ADA assay result and negative mADA assay result = pre-Ab positive either from the patient or from PE.
Note that in the Phase 2 TITAN study, the incidence of pre-dose pre-Ab positive samples are known because pre-dose clinical samples are tested for ADA using the conventional ADA assay.

2.) Positive conventional ADA assay result and positive mADA assay result = TE ADA positive.
Note that the incidence of TE ADA from TITAN and HERCULES are reported in the proposed drug labeling.

Reviewer's Comment: *The Applicant's three-tiered approach following the guidance to evaluate ADA and TE ADA for the pivotal clinical studies is adequate. Although the mADA assay may underestimate the incidence of TE ADA compared to the conventional ADA assay, as discussed in the review below, all positive samples based on the conventional ADA assay are tested for NAb in the phase 3 study.*

We consulted with the OBP Immunogenicity Working Group regarding the acceptability of the Applicant's strategy for detection of anti-drug antibodies (ADA) and treatment-emergent ADA (TE-ADA)

on 11/14/2018 and 11/21/2018. Based on the discussion, we recommended to the review team that the Immunogenicity section of the label should include a discussion around the presence of baseline positive samples (i.e., pre-existing antibodies as referred to by the Applicant), which were found to be present in 55 of 97 subjects in the caplacizumab treatment group and 46 of 73 subjects in the placebo arm of the phase 3 HERCULES study. (b) (4) complete description of the immunogenicity profile of the product.

Anti-drug Antibody Binding Assays:

Reviewer's Comment: *The Applicant submitted three sets of ADA binding assays (in Section 5.3.1.4). The following reviewer-generated table compares summary information of the ADA assays:*

Table 1: ADA binding assays

ADA Assay (Assay Validation report #)	Usage (Phase)	Assay Format	Developer and Testing Site	Labeled Reagents	Caplacizumab conc. for confirmatory assay	Minimum Required Dilution (MRD)
ELISA (07/029-012, 07/029-015, 07/029-043, AYX096EL-090963B)	1, 2	Bridging Assay	(b) (4)	Biotinylated caplacizumab	100 µg/ mL	4
Conventional ECL Assay (AMV-0014-MVR, AMV-0040-MVR)	1, 2, 3	Bridging Assay	Ablynx-GLP	Biotinylated and SULFO-tagged caplacizumab	10 µg/ mL	96
Modified ECL Assay (A068-C-2014-007, AMV-0041-MVR)	2, 3	Bridging Assay	Ablynx-GLP, Ablynx-R&D	Biotinylated caplacizumab and SULFO-tagged alanine-extended caplacizumab	10 µg/ mL	100

All three sets of assays follow the bridging assay format. In each ECL assay set, the same assay platform was used as the screening, confirmatory, and titer assay. In the ECL confirmatory assay, antibodies are tested for target specificity using competition with caplacizumab. In the titer assay, samples are diluted 32-fold followed by 2-fold serial dilution until dropping below the titration cut-point.

The ADA assays were used to support the following clinical studies (Information derived from Tables 28, 29, and 32 and Section 7.2.3.7 of the *Immunogenicity Risk Assessment Report No 24_A0068_10_0292 v5.0* of the submission):

Summary of ADA assay results for clinical studies

Phase and ADA assay verification reports	Description of role in study	Subjects positive for pre-Ab[1]	Subjects positive for ADA based on ADA assay	Subjects positive for TE ADA based on mADA assay
Phase 2 ALX0681-2.1/10 (TITAN) Validation: AMV-0014-MVR mADA Validation: A068-C-2014-007	Assay adapted to improve drug tolerance	Placebo: 3/37 Treatment: 6/35[2]	Placebo: 15/30 Treatment: 4/35	Placebo: 0/35 Treatment: 3/35

Phase and ADA assay verification reports	Description of role in study	Subjects positive for pre-Ab[1]	Subjects positive for ADA based on ADA assay	Subjects positive for TE ADA based on mADA assay
Phase 1 ALX0681-C102 Validation: AMV-0014-MVR	Assay in support of healthy volunteer study evaluating the bioequivalence of the current formulation intended for marketing with the liquid formulation used in initial studies	1/24	0/24	Not used
Phase 3 ALX0681-C301 (HERCULES) Validation: AMV-0040-MVR mADA Validation: AMV-0041-MVR	Assay optimized in support of this study	Placebo: 46/73 Treatment: 55/97[3]	Same as subjects positive for pre-Ab	Placebo: 1/73 Treatment: 3/97
[redacted]				(b) (4)

[1]Some patients were not pre-dose sampled. [2]These pre-Ab values are derived from conventional ADA assay results using pre-dose samples only [3]All pre-Ab positive samples were tested positive with the ADA assay but not the mADA assay.

Reviewer's Comment: *In the following sections, only the validation report for the most recent versions of the ECL assays are reviewed. The ELISA was only used in early phase studies because it had low drug tolerance and therefore its assay validation reports were not reviewed.*

Conventional ADA Assay: ECL Assay

Only the second validation report, AMV-0040-MVR, is reviewed because it supports the assays used in the phase 3 clinical studies.

Method

The conventional ADA screening assay is an ECL based bridging assay. Briefly, human plasma samples are incubated overnight with a 1:1 mixture of biotinylated and SULFO-tagged caplacizumab (master mix) to form immune complexes containing ADA bound to the tagged caplacizumab molecules. The formed immune complexes are captured via the biotinylated caplacizumab on a MSD GOLD 96-well Streptavidin SECTOR plate and quantified by detection of the ECL signal from the SULFO-tag using a MESO QuickPlex SQ120 instrument. The confirmatory and titration assays use the same ECL based bridging format. Samples for the confirmation assay are tested with or without 10 µg/ mL caplacizumab spiked in the master mix.

Reviewer's Comment: *The ECL-based assay does not incorporate any procedure to enhance sensitivity or specificity, such as acid dissociation or sample purification.*

Essential Reagents and Concentrations

Positive Controls (PC):

Table 2: Positive Control samples and assay sensitivity

Name	Type of Reagent	Animal Source	Batch Number	Sensitivity in healthy human serum (LOD)(ng/mL)
(PC1) pAb anti-DP rabbit pool (consisting of Rb24966 & Rb24967) (Used in Quality Control samples)	Polyclonal pool	Rabbit	F(542)/160318/1	114.4 and 87.5 (95% and 50% Confidence Interval (CI))
(PC2) mAb anti-DP ABH0070	Monoclonal	Mouse	B8#20140211r1.A6-A10	44.4 (50% CI)
(PC3) mAb anti-DP ABH0014	Monoclonal	Mouse	G-SLI-146-ABH0014-A9080115	25.1 (50% CI)
(PC4) pAb anti-DP Rb6869 (Also used in first validation report)	Polyclonal	Rabbit	P(025)/110302/1_2	48.5 (50% CI)
(PC5) mAb neutralizing anti-DP ABH0066	Monoclonal	Mouse	G_SLI_163_ABH0066_B10116	1392.6 and 539.5 (95% and 50% CI)

Sensitivity (LOD) in serum from TTP patients: Using PC1: < 25 ng/mL (50% CI)

Sensitivity (LOD) in serum from healthy adolescents: Using PC1: 38 ng/mL (50% CI)

Low Positive Control (LPC): 137 ng/mL (Based on 99% CI, referred to as LoQC1)

191.9 ng/mL (Based on 99.9% CI, referred to as LoQC2)

LoQC1 and LoQC2 calculated based on 6 LOD determinations using PC1 and PC2.

Note: After rounding and aligning with parameters of the modified ADA assay, LoQC1 and LoQC2 were redefined as LoQC3 = 140 ng/mL and LoQC4 = 190 ng/mL. Quality control samples containing ADA at the LoQC and HiQC (20000 ng/mL) concentrations were prepared using PC1 in healthy human sera.

High Positive Control (HPC): 20,000 ng/mL

Negative Control (NC): Pooled human serum (PHuS) Batch # PHuS#13SEP2016_1

Additional sera: Pre-existing (pre-Ab) depleted pooled human serum, healthy individual human serum (adult), healthy individual and pooled human serum (adolescent), individual and pooled TTP patient human serum. Note: pre-Ab was depleted by using affinity chromatography.

Master mix: 1 µg/mL ALX-0081-Bio, 1 µg/mL ALX-0081-Sulfo (1:1 tagged caplacizumab mix). Note: caplacizumab in the master mix and used as a drug in the drug displacement assay was derived from reference standard batch ALX-00810RS-04

Minimal Required Dilution (MRD): 1/96 after dilution in the master matrix (1/32 dilution in assay diluent followed by 1/3 dilution in master mix). The MRD was established during method development and confirmed in

healthy adult, healthy adolescent, and TTP patient sera versus buffer (>80% recovery for all samples spiked with HiQC, LoQC4, and LoQC3).

Screening and Titration Cut Point Determination

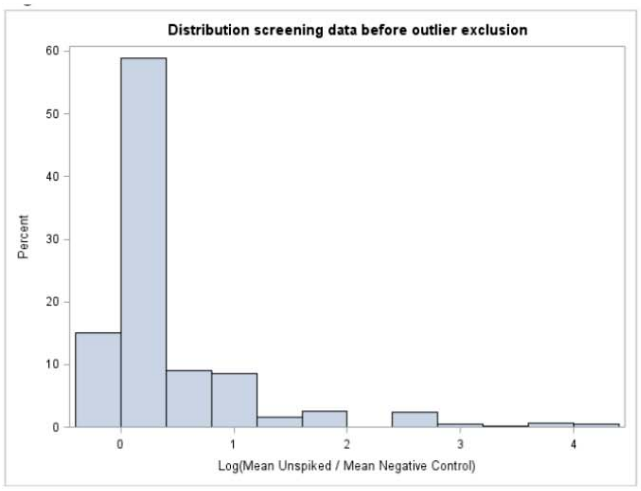
The screening, confirmatory, and titration cut-points were determined in parallel by running screening and confirmatory assays simultaneously on the same plate using a balanced experimental design. The cut-point determination in serum samples from healthy adults was based on data from 420 samples from 70 individual samples generated from 6 runs by 2 different operators over 3 days. The cut-point determination in serum samples from healthy adolescents was based on data from 180 samples from 45 individual samples generated from 4 runs by 2 different operators over 2 days. The Applicant chose to determine **floating** screening and titration cut points while the confirmatory cut point is **fixed**, based on the ratio of the sample's caplacizumab-spiked ECL value over its respective unspiked ECL value, and transformed as a % inhibition of the signal in the confirmatory assay.

Reviewer's Comment: *The experimental design for determination of cut points is acceptable.*

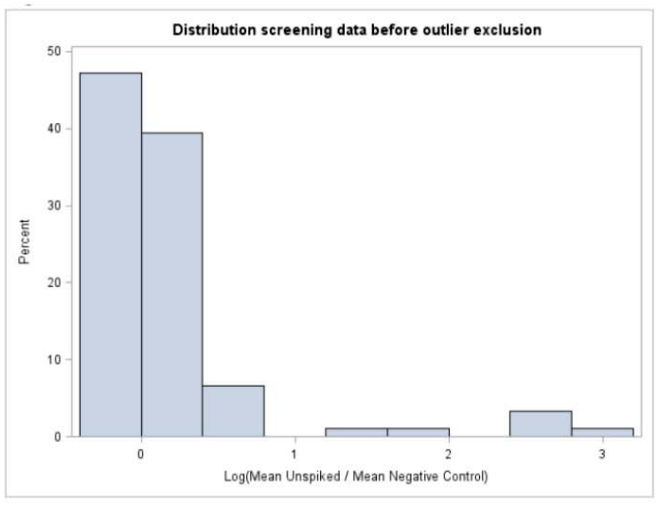
To minimize between plate variation, the Applicant chose to normalize ECL responses by the mean of the NC of the corresponding plate. The ratio was log-transformed. Suitability of the NC was assessed by determining whether ECL signals from NC drift with the unspiked individual subject samples. It was determined that the NC trends positively with the individual samples with a significant correlation.

Outlier exclusion was based on an iterative process using a mixed-effects model and Tukey's outlier criterion (OD <Q1 – 1.5IQR or >Q3+1.5*IQR where Q1 and Q3 are defined as the ODs at which 25% of the population had lower or higher ODs, respectively IQR is defined as Q3-Q1). The outlier selection process was performed on log-transformed response data normalized by the mean of the negative controls of the corresponding plate as shown in *Figure 7 of Annex 4: 24_A0068_17_0021 of Validation Report AMV-004*0 (adult samples) and *Figure 7 of Annex 5: 24_A0068_17_0023 of Validation Report AMV-004*0 (adolescent samples) of the submission:

Adult Samples



Adolescent Samples



For the adult human samples, outlier exclusion was performed over 7 iterative rounds that removed 43 analytical outliers. The mixed-effects model was fit to the remaining data and the subject best linear unbiased predictions that meet Tukey's criteria were considered as biological outliers (19 outliers over 4 model fitting rounds). In total, 132 adult samples were identified as outliers, retaining 288 samples. Similarly, a total of 63 adolescent samples were identified as outliers, retaining 117 samples (4 analytical outliers and 15 biological outliers).

Reviewer's Comment: *Per USP38 <1106.1> "Data from the scale (e.g., original, log) that provides the most symmetric or close-to-normal distribution should be used in all subsequent analyses, such as outlier evaluation, cut-point calculations, and comparisons of means and variances across assay runs." However, the log transformed data before outlier exclusion does not look normally distributed. Furthermore, the Shapiro Wilk test results presented in Table 1 of Annex 4: 24_A0068_17_0021 of Validation Report AMV-0040 (adult samples) and Table 1 of Annex 5: 24_A0068_17_0023 of Validation Report AMV-0040 (adolescent samples) of the submission revealed that log transformation did not normalize the data:*

Adult data:

	Untransformed Ratios	Log-transformed Ratios
p-value Shapiro-Wilk test	<0.0001	0.0017
Skewness	1.87	0.81

Adolescent data:

	Untransformed Ratios	Log-transformed Ratios
p-value Shapiro-Wilk test	<0.0001	0.0002
Skewness	1.32	1.19

Application of Tukey's outlier criterion on very skewed data may explain the high number of outliers since Tukey's method works under the assumption that the data has a normal distribution. However, considering the high incidence of pre-Ab in healthy subject sera, and that the Applicant ultimately used a non-parametric approach to define the cut point for healthy adult sera, as described below, this is acceptable.

The log transformed and outlier excluded data remained skewed for the adult samples (Shapiro-Wilk p-value < 0.0001, skewness=0.723) but were normally distributed for the adolescent samples (Shapiro-wilk p-value = 0.36, skewness = 0.35). Therefore, for the adult samples, a robust alternative approach based on the median was taken to determine the screening and titration cut-point normalization factors by using the formula:

*exp(median + k*1.4826*MAD) with k » 1.645 (one-sided 0.05 significance level) (**Screening cut-point**), k » 3.09 (one-sided 0.001 significance level) (**Titration Cut-point**), and k=6 respectively (**Titration Cut-point based on median + 6*1.4826*MAD**). MAD = median absolute deviation*

For the adolescent samples, a parametric approach was taken to determine the normalization factors:

*exp(mean + k*SD) with k ≈ 2.33 (one-sided 0.05 significance level, corresponding to a 5% false positive rate (**Screening cut-point**), k ≈ 3.09 (one-sided 0.001 significance level), and k=6 respectively (1 % false positive rate) (**Titration cut-point**).*

The results are summarized below:

Samples	Screening cut-point normalization factor (NF)	Titration cut-point NF
Healthy Adults	1.32	1.556 (0.001 significance) and 2.168 (calculated by median)
Healthy Adolescents	1.156	1.322 (0.001 significance) and 1.731 (calculated by median)

The above calculations were repeated on a dataset in which subjects with pre-Abs are excluded. Subjects with a %inhibition exceeding the confirmatory cut-point (41.7%) were considered to have pre-Abs. The newly calculated screening cut-point normalization factor (1.234) and confirmatory cut-point (50.06%) are comparable to the cut points determined using the non-pre-Ab excluded dataset [1.32 and 44.75% (refer to below)]. Therefore, the Applicant chose not to incorporate exclusion of subjects with pre-Abs.

Reviewer's Comments:

Screening cut-point determination:

Using the screening cut point factor of 1.32 determined by the Applicant, the Reviewer found that 29/420 (6.9%) healthy subject samples are screened positive but were not confirmed positive, and 101/420 (24%) healthy subject samples are screened and confirmed positive, indicating the presence of pre-Ab (Raw data in Table 18 of Annex 4 in the validation report AMV-0040-MVR). Although the false positive rate (6.9%) is higher than the targeted 5%, it is within the guidance recommendation of 90% one-sided lower confidence interval for the 95th percentile of the negative control population. Therefore, the screening cut point factor of 1.32 is acceptable.

However, samples from different populations can have different background activity in ADA assays. The cut point is determined using plasma samples from healthy subjects. Per the guidance, the Applicant should confirm that the cut point determined during assay validation is suitable for the population being studied, i.e. aTTP patients. The Applicant did not confirm the cut-points in the validation with patient sera because sera from patients of this rare disease is not readily available. The Applicant indicates that (b) (4) *Furthermore, other validation parameters such as sensitivity and precision were evaluated using both healthy and TTP patient sera. This is acceptable.*

Titer cut-point determination:

After reviewing the titer precision data, the proposed titer cut point (1.556 x mean of NC) is deemed acceptable.

Confirmatory Cut Point Determination

The results of the same healthy human plasma samples used for screening cut-point determination analyzed in the absence and presence of excess caplacizumab (10 µg/mL) were used for the calculation of

the confirmatory cut-point. An inhibition of the sample's mean ECL value in the presence of caplacizumab was calculated as follows:

% Signal Inhibition = 100 (1-[spiked sample mean ECL / unspiked sample mean ECL]).

The confirmatory cut-point was determined on the log-transform of the mean drug spiked/mean drug unspiked ratio data (log (SI/US)), which was normally distributed (Shapiro Wilk test p-value = 0.90, skewness = -0.84). Outliers were determined by a mixed-effects model in an iterative manner, as described above for the screening cut-point determination. Altogether 30 analytical outlier adult subjects and 20 biological outlier adult subjects were identified, constituting a total of 142 outlier samples that were excluded, retaining 278 samples for confirmatory cut-point determination. Similarly, a total of 28 adolescent samples were identified as outliers, retaining 152 samples. The parametric confirmatory cut-point was determined for the adult dataset because it is normally distributed while the robust alternative confirmatory cut-point was determined for the adolescent dataset because it deviated from normality (Shapiro-Wilk p-value <0.0001):

Samples	Confirmatory Cut-point (1% false positive rate)	Confirmatory Cut-point (0.1% false positive rate)
Healthy Adults	50.06% inhibition	53.23% inhibition
Healthy Adolescents	54.18%	50.05%

The Applicant reported that the % signal inhibition is ~ 68% (aTTP patient serum) and ~60% (healthy adult serum) for LoQC (137 ng/mL), and 96.48% (aTTP patient serum) 96.22% (healthy adult serum) for HPC at 20,000 ng/mL.

Reviewer Comments*: The statistical analysis for confirmatory cut point determination is appropriate. Furthermore, the confirmation cut-point is able to positively confirm ADA in both healthy and aTTP patient serum spiked with LoQC amount of ADA.*

Assay Precision

Quality control (QC) samples consisting of healthy human sera spiked with LoQC1, LoQC2, LoQC3, LoQC4, and HiQC concentrations were prepared using PC1. To evaluate the precision of the QC and negative control (NC) samples, 18 test runs of QC and NC samples were evaluated by 3 different analysts. The %Coefficient of Variance (CV) for intra-run precision was <5% for all QC and NC samples. The %CV for inter-batch precision was <15% for all QC and NC samples. Note that the ECL values are slightly higher than the plate-specific SCPs (right-most column in table below) for the LoQC4 (190 ng/mL), which indicates that the selected level of LoQC3 (130 ng/mL) is only slightly higher than the assay sensitivity:

Table 24 (from the Validation Report AMV-0040 of the submission):

		Response				
Level (ng/mL)	Run n°	Start	Middle	End	Mean NC	Plate Specific cut-point
LoQC4 190.0	VB11_1	239.0	247.0	252.0	146.3	193.2
	VB12_1	280.5	264.0	259.5	161.3	213.0
	VB13_1	248.5	266.5	248.5	140.7	185.7
	VB14_1	268.5	282.0	269.0	172.5	227.7
	VB15_1	242.5	249.0	241.5	137.8	181.9
	VB18_1	240.5	249.0	261.5	147.7	194.9
	VB20_1	265.5	278.5	278.0	169.5	223.7
	VB21_1	233.5	247.5	246.5	130.7	172.5
	VB22_1	245.5	251.5	276.5	162.8	214.9
	VB23_1	276.0	268.0	283.5	197.2	260.3
	VB24_1	250.0	277.5	259.0	160.2	211.4
	VB26_1	270.5	256.0	262.5	136.7	180.4
	VB27_1	207.5	209.5	210.0	121.8	160.8
	VB29_1	232.0	231.0	238.0	140.3	185.2
	VB30_1	219.0	224.0	217.0	119.7	158.0
	VB31_1	223.0	208.5	270.5	119.2	157.3
	VB32_1	234.0	239.0	247.0	135.7	179.1
	VB34_1	233.5	240.5	225.5	133.3	176.0

All HiQC and LoQC4 samples screened positive and LoQC3 screened positive in 98.1% of the cases. To evaluate the precision of the scoring in the confirmatory assay, the QC samples were analyzed in the presence or absence of the drug. An average percent inhibition 96.55%, 55.53%, 59.48% and 30.07% was observed for the HiQC, LoQC3, LoQC4, and NC respectively. Since LoQC4 confirmed positive in all cases, the Applicant indicated that LoQC4 can be used as the LoQC level during sample analysis. This was confirmed in matrix interference assessments, in which the QC samples were prepared in 5 samples from healthy adults, 5 samples from healthy adolescents, and 10 samples from aTTP patients. All QC samples were screened and confirmed positive except for LoQC4 for 2 healthy patient samples. From these data, the Applicant concluded that LoQC4 (190 ng/mL) and HiQC (20,000 ng/mL) concentrations are appropriate to monitor assay performance during sample analysis.

Reviewer's Comments:
The assay precision is acceptable.

Assay Suitability Control

The in-study acceptance criteria were established using data from all precision runs (3 analysts) and NC samples from 99 validation runs. The lower and upper limits for the QC samples are based on a 0.5% failure rate while the upper limit for the NC is based on a 1% failure rate using Student's t-distribution as summarized below (from the submission):

Lower limit normalized QC	Normalized QC value	Upper limit Normalized QC
58.1	≤ HiQC ≤	125.5
1.3	≤ LoQC3 ≤	1.8
1.3	≤ LoQC4 ≤	2.1

	Upper limit NC (ECL)
NC (ECL) ≤	189.9

Reviewer's Comments:
The approach to establish the in-study acceptance criteria for reagents is acceptable.

Assay Sensitivity

The assay sensitivity (LOD, Table 2) was verified for each of the different positive controls diluted in healthy human serum. For affinity purified pAb Rb pool and mAb ABH0066, 6 independent batches were evaluated whereas for the other positive control samples, 1 batch was evaluated. Additionally, for PC1, sensitivity was determined in aTTP serum and in healthy adolescents as reported above. The sensitivities were determined by interpolating the concentration of the positive control antibody corresponding to the SCP by fitting the concentration response curve using a second order polynomial fit (quadratic) without weighting (PC1-4) and with a linear regression model (PC5).

Reviewer's Comments:

The sensitivity of the ADA assay is <100 ng/mL (50% CI) using PC1, PC2, PC3 and PC4 in normal adult, normal adolescent, and in TTP patient sera. Only PC1 is used in the preparation of QC samples. Based on results for assay precision, the LOQ of the assay was determined to be LoQC4 (190 ng/mL). The approach used to determine assay sensitivity is appropriate.

Drug Tolerance

The Applicant evaluated the ability of the assay to detect ADA (PC1, PC2, PC3) in the presence of circulating drug and established drug tolerance limits for the assay. The result is provided in the following Tables from the submission:

PC1:

	Interpolated sensitivity at different concentrations drug			
	Concentration drug ALX-0081-RS-04			
	10.0 µg/mL	5.0 µg/mL	1.0 µg/mL	0.0 µg/mL
Slope (y1 - y2)/(X1 - X2)	5.3	2.5	4.7	N/A
intercept y1 - ((slope)*X1)	-885.6	-312.5	-733.6	N/A
(Interpolated) sensitivity y = (slope)*x + intercept	257.7 ng/mL	224.9 ng/mL	270.7 ng/mL	<140.0 ng/mL

PC2:

	Interpolated sensitivity at different concentrations drug			
	Concentration drug ALX-0081-RS-04			
	10.0 µg/mL	5.0 µg/mL	1.0 µg/mL	0.0 µg/mL
Slope (y1 - y2)/(X1 - X2)	N/A	1.4	0.9	N/A
intercept y1 - ((slope)*X1)	N/A	-159.4	-85.9	N/A
(Interpolated) sensitivity y = (slope)*x + intercept	<140.0 ng/mL	208.0 ng/mL	142.4 ng/mL	<140.0 ng/mL

PC3:

	(Interpolated) sensitivity at different concentrations drug			
	Concentration drug ALX-0081-RS-04			
	10.0 µg/mL	5.0 µg/mL	1.0 µg/mL	0.0 µg/mL
(Interpolated) sensitivity (ng/mL)	34.2 ng/mL	39.0 ng/mL	38.6 ng/mL	23.0 ng/mL

The Applicant concludes that in the presence of the 10 µg/mL caplacizumab, sensitive detection of ADA is still possible. Therefore, they claim the assay is drug tolerant up to a level of 10 µg/mL drug.

Reviewer's Comment:

According to the clinical pharmacology reviewer Robert Schuck, the mean level of caplacizumab in the subjects from the study ALX0681-C301 ranged from 443 ng/mL to 529 ng/mL from week 1 to week 9 with minimums and maximums of 111 ng/mL to 1640 ng/mL. To understand the sensitivity of the assay in the presence of drug, the Applicant determined the amount of drug that could be present in plasma and still allow for the detection of three positive controls at concentrations ranging from 20,000 ng/ml to 140 ng/mL (PC1, PC2) or to 10 ng/mL (PC3). The results presented above indicate that at the expected drug plasma concentration (~0.1 – 1.6 µg/mL) the assay sensitivity with PC1, which is used to analyze in-study samples, may be >270.7 ng/mL, which is > LOQC4 (190 ng/mL). According to the assay protocol, the samples undergo an MRD of 1/96 dilution, effectively lowering the potential drug concentration to ~0.001-0.016 µg/mL. Overall, assay sensitivity in the presence of on-board drug is acceptable.

Titer Precision

Titer calculation is based on linear regression of the dilution factor corresponding to the response values directly below and equal to or above the titer cut-point. Titer precision and minimum significant ratio (MSR) was determined for healthy adult sera (4%, 2.04), healthy adolescent sera (3.3%, 1.94) and aTTP patient sera (3.2%, 1.73).

Reviewer's Comments:

The approach used to determine the precision of the titer assay is appropriate.

Stability

Stability of quality control samples HiQC, LoQC3, and LoQC4 was evaluated after freeze/thaw cycles (10, 8, 6, 4, 2, 1 cycles), storage for 24 hours at room temperature (22 ± 3 ºC), storage for 3 hours at ultrafreezer temperature (-70 ± 10 ºC), storage for 24 hour in the refrigerator (5 ± 3 ºC), or long term storage at ultrafreezer or freezer (-20 ± 5 ºC) temperatures (6, 12, 18, 24 months). All samples tested had >80% recovery and <20% intra-run precision.

Reviewer's Comment:

The quality control samples are stable under the studied conditions.

Specificity of Confirmatory Assay

The specificity of the confirmatory assay was assessed by spiking ovalbumin, an irrelevant protein with similar molecular weight as the DP, to the master mix at the same concentration as the DP. Addition of ovalbumin did not lead to % inhibition higher than the confirmatory cut-point in all quality control samples tested.

Reviewer's Comment:

The specificity of the confirmatory assay is acceptable.

Robustness

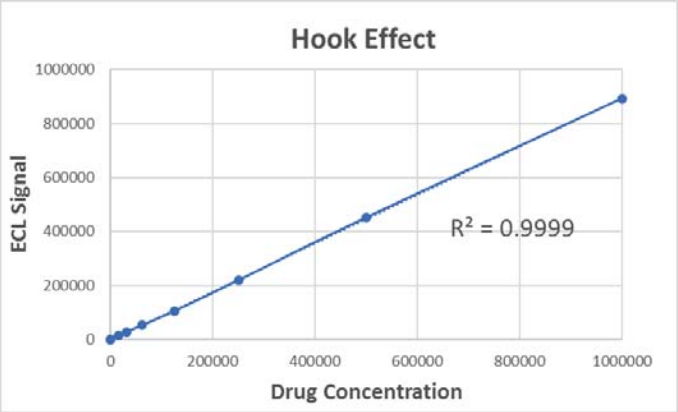
Robustness of the assay was evaluated by assessing inter-operator variability (2 different operators), assay drift (Assays with quality control samples performed 3 times in technical duplicate per plate) and influence of incubation time (Assays with quality control samples performed with ±10% incubations times). The results indicate that assay variability under these tested conditions did not affect the integrity of the data.

Reviewer's Comment:

The robustness of the assay is acceptable.

Hook Effect

To assess whether there is a hook effect, the Applicant measured the ECL signal from the serial dilutions of PC1 beginning at 1 mg/mL (Table 37 of Validation Report AMV0040). The data is plotted below by the reviewer:



Reviewer's Comment:

The hook effect was not observed for this assay using PC1.

Selectivity – Matrix interference and Target interference

Matrix interference was assessed by spiking PC1 at HiQC, LoQC3, and LoQC4 into hemolytic pooled human samples and lipemic pooled human samples, which are then analyzed by the assay. For all samples, the in-study acceptance criteria and intra-run %CV were met. Target interference was assessed by spiking a serial dilution of the target vWF starting at 60,000

ng/mL (100% serum level) into negative samples. From an interpolated vWF concentration of 53,866 ng/mL onwards, a false positive signal was detected. The Applicant notes that the commercial vWF may not be fully representative of the native vWF present in patients, and that the presence of false positives due to elevated target levels will be assessed by correlating the measured target levels with ADA reactivity.

Reviewer's Comment:

Selectivity was appropriately evaluated.

Modified ADA Assay: ECL Assay

Only the second validation report, AMV-0041-MVR, is reviewed because it supports the assays used in the phase 3 clinical studies.

The mADA screening assay is an ECL based bridging assay that is nearly identical to the ADA screening assay described and reviewed above. The Applicant claims that the mADA assay is more sensitive towards treatment emergent (TE) ADA, and is used on samples that were confirmed positive by the ADA assay to determine the TE ADA positive samples as summarized above. The clinical studies supported by the mADA assay are also summarized above.

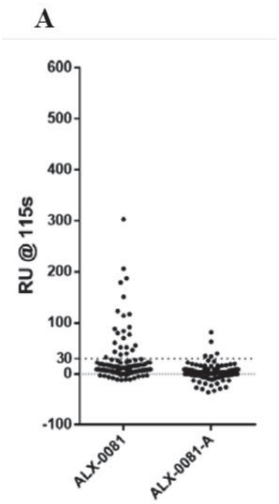
Briefly, human plasma samples are incubated overnight with an excess of biotinylated caplacizumab and SULFO-tagged alanine-extended caplacizumab (master mix) to form immune complexes. The formed immune complexes are captured via the biotinylated caplacizumab on a MSD GOLD 96-well Streptavidin SECTOR plate and quantified by detection of the ECL signal from the SULFO-tag using a MESO QuickPlex SQ120 instrument. The confirmatory and titration assays use the same ECL based bridging format. The assays do not incorporate any procedure to enhance sensitivity or specificity, such as acid dissociation or sample purification.

Reviewer's Comment:

The primary difference between the mADA and the ADA assay is the use of the SULFO-tagged alanine-extended caplacizumab in the master mix of the mADA assay. However, the Applicant initially did not provide scientific justification to support the claim that the DP with an additional C-terminus alanine has reduced binding to most pre-Ab, and that most TE ADA would not recognize the C-terminus region. It is possible that some TE ADA would bind to the C-terminus region, potentially underestimating the incidence of TE ADA. In response to an IR sent on 11/8/2018, the Applicant provided data and scientific justification in support of the suitability of the modified DP (eCTD 0021) as summarized below:

- The location of pre-Ab binding was studied using surface plasmon resonance (SPR) with different DP variants and pre-Ab purified from pre-Ab-positive donors. It was observed that DP variants with C-terminal tags such as c-myc-His6-tag or FLAG3-His6-tag exhibited diminished or no binding to purified pre-Ab. The effect of adding one or a few amino acids at the C-terminus

was then studied and it was observed that the addition of a single alanine showed decreased binding to immobilized pre-Ab. Additionally, the modified DP with a single C-terminus alanine (ALX-0081-A) had reduced binding to pre-Ab in human sample sets biased for samples presenting with pre-Ab (100 serum and 16 plasma samples) relative to unmodified DP (ALX-0081) as shown in Figure 2 of Section 1.11.3 SN0021 RfI Clinical Pharmacology of the submission and reproduced to the right. Binding was measured using SPR analysis, and the response units reported indicate the response at the peak of the association curve (115 seconds). Similar observations were made for a panel of unmodified and alanine extended nanobodies developed by the Applicant at both 115 seconds and 800s (close to the bottom of the dissociation curve).



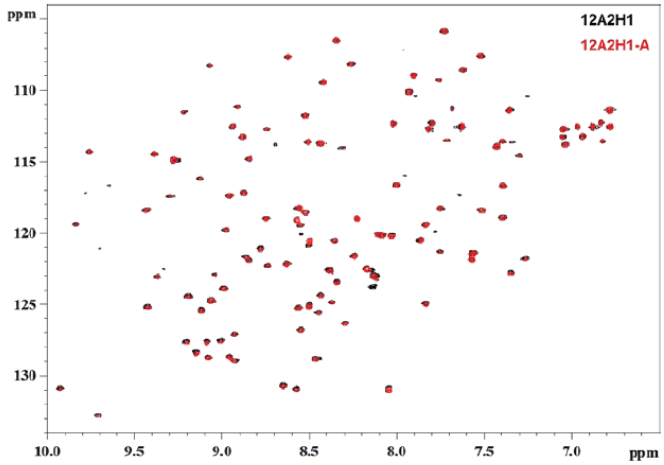
Note that the response units at 800s may be a more appropriate indicator of the suitability of the C-terminus alanine because the wells in the assay are washed prior to exposure to the detection reagents and therefore would be sensitive to dissociation rates.

Reviewer's Comment: *The provided data indicate that the modified DP has reduced affinity towards pre-Ab from human subjects. Although pre-Ab towards other epitopes may still bind to the modified DP, such pre-Ab appears to be rare in the pre-Ab samples that were studied.*

However, TE ADA that bind to the C-terminus region would potentially not bind to the modified DP. Therefore, the mADA assay could underestimate the incidence of TE ADA. It may be unlikely for ADA targeting the C-terminus region to be neutralizing since the C-terminus region is distal from the binding region. TE ADA could also impact PK, as was observed for subjects (b) (6) *with TE ADA in the HERCULES study (Refer to the Immunogenicity Risk Assessment in the submission). Overall, the risk to the patient is low since all patients receive immune suppressors, and since negative impact to efficacy from ADA has not been observed in any of the studies.*

- *The Applicant obtained ^{1}H-^{15}N HSQC NMR spectra of (1) a single domain of the DP and (2) a single domain of the DP with the addition of one c-terminus alanine, which is comparable to the modified DP used in the mADA assay. ^{1}H-^{15}N HSQC spectra provide the correlations between the nitrogen and amide proton on each amide of the protein, which is influenced by the unique chemical environment around each amide. Therefore, these spectra can be interpreted as fingerprint patterns of higher order structures. The spectra of the single domain of the DP and single domain of the modified DP are of high quality and appear to be highly comparable as shown below (from Figure 9 in Section 5.3.1.4 Study Report A-0068-00-TIM-SC-15-PSR-218 – Study No: A-0068-00-TIM-SC-15-PSR-218):*

Figure 9: Overlay of the 12A2H1 (black) and 12A2H1-A (red) 1H-15N HSQC spectra.



Differences between the spectra appear minor and are predominantly associated with residues proximal to the C-terminus.

Reviewer's Comment: *Dr. Ksenija Grgac, a full-time reviewer in OBP Division IV with expertise in NMR has examined the spectra. She confirms that the spectra are of high quality and appear comparable.*

Because the assay format and validation strategy of the mADA and ADA assays are nearly identical, including identical positive controls and serum samples used for validation, please refer to the review of the ADA assay on the validation approach. The validation results for the mADA are summarized below in a reviewer generated table. The ADA assay results are included to aid in direct comparison:

	ADA binding assay	**Modified ADA binding assay**
Screening Cut Point	NF=1.32 (5% FP)	NF=1.167 (5% FP)
Confirmatory Cut Point	50.06% (1% FP)	41.7% (1% FP)
Titer Cut Point	NF=1.556 (1%FP)	NF=1.298 (1%FP)
Quality Control: HiQC	20,000 ng/mL	20,000 ng/mL
Quality Control: LoQC	190 ng/mL	140 ng/mL
Sensitivity	87.5 ng/mL (PC1, 50% consistency)	37.9 ng/mL (PC1, 50% consistency)
Intra-run precision	HiQC: 4% LoQC: 4.6% NC: 4.6% (AC: (b) (4)%)	HiQC: 3.9% LoQC: 3.8% NC: 5.4% (AC: (b) (4)%)
Inter-batch precision	HiQC: 13.8% LoQC: 7% (AC: (b) (4)%)	HiQC: 17% LoQC: 19% (AC: (b) (4)%)
Drug tolerance	in 1 µg/mL drug = 270.7 ng/mL	in 1 µg/mL drug < 85 ng/mL
Minimum Significant Ratio	Healthy sera: 2.04 (4% precision), patient sera: 1.73 (3.2% precision)	Healthy sera: 2.13 (3.8% precision), patient sera: 2.57 (4.8% precision)
Minimum Required Dilution	1/96 dilution	1/96 dilution

NF: Normalization Factor: FP: False positive rate as established by a t-distribution AC: Acceptance Criteria

Reviewer's Comment: Overall, the validation results indicate that the mADA assay is a more sensitive and drug tolerant assay than the ADA assay. Furthermore, the drug tolerance study indicates that the assay can consistently detect LoQC concentrations in the presence >1 µg/mL DP, which is greater than the observed amount of DP in patient samples as mentioned above.

Neutralizing Antibody Assays:

Reviewer's Comment: *The Applicant submitted information on two neutralizing antibody (NAb) assays (in section 5.3.1.4). The following reviewer-generated table compares summary information of the NAb assays:*

Table 2: NAb assays

NAb Assay (Assay Validation report #)	Usage (Phase)	Assay Format	Developer and Testing Site	Labeled Reagents	Minimum Required Dilution (MRD)
Functional NAb Assay (11/029-060, AMV-0044-MVR	2, 3	Functional Assay (ELISA)	Ablynx-GLP	Polyclonal Rabbit Anti-Human vWF-HRP	2.3
Alternative NAb Assay (AMV-0042-MVR)	3	Bridging Assay	Ablynx-GLP	Biotinylated and SULFO-tagged caplacizumab	96

The functional NAb assay directly measures the presence of NAbs in samples by their ability to inhibit the DP's mechanism of action. The alternative NAb assay detects and quantifies the ADA in samples that requires interaction with the CDR loops of the DP. Both assays are two-tiered, with a screening assay and a titer assay. Because the functional NAb assay is not drug tolerant, it is only used to assess ADA positive samples from pre-dose and washout time points. All other samples are analyzed with the alternative NAb assay. The NAb assays were used to support the following clinical studies as summarized by the reviewer (Information derived from Table 28 and Section 7.2.3.7 of the *Immunogenicity Risk Assessment Report No. 24_A0068_10_0292 v5.0* of the submission):

Phase and ADA assay verification reports	Subjects positive for pre-Ab[1]	Subjects positive for ADA based on ADA assay	Subjects positive for TE ADA based on mADA assay	Subjects positive for NAb
Phase 2 ALX-0081-2.1/09 Functional NAb Validation: 11/029-060 (ADA tested only in Treatment Group)	Treatment: 21/171[2]	Treatment: 35/174 (no confirmatory) TE ADA from increase in titer: 17/174	N/A	0
Phase 3 ALX0681-C301 (HERCULES) Functional NAb Validation: AMV-0044-MVR Alt NAb Validation: AMV-0042-MVR	Placebo: 46/73 Treatment: 55/97[3]	Same as subjects positive for pre-Ab	Placebo: 1/73 Treatment: 3/97	**Placebo:** NAb assay: 1/73 Alt NAb assay: 0/73 **Treatment:** NAb assay: 2/97 Alt NAb assay: 4/97
Ongoing Phase 3 ALX0681-C302 Functional NAb Validation: AMV-0044-MVR Alt NAb Validation: AMV-0042-MVR	N/A	N/A	N/A	N/A

[1]Some patients were not pre-dose sampled. [2]These pre-ADA values derived from ADA assay results using pre-dose samples only [3]All pre-Ab positive samples were tested positive with the conventional ADA assay but not the mADA assay.

Reviewer's Comment: *In the following sections, only the validation report for the most recent versions of the NAb assays are reviewed.*

Functional NAb Assay:

Method

The NAb screening assay is an ELISA based functional assay. Briefly, human plasma samples first undergo complement inactivation by heating at 56ºC for 30 minutes followed by 10 min centrifugation (13400 x g) to overcome matrix interference. The inactivated samples are subsequently incubated with Protein G and acid eluted to enrich for antibodies. Upon pH neutralization of the eluates, the samples are incubated with the DP followed by further incubation with vWF and ristocetin, which activates vWF for binding to blood platelets. The mixture is then applied to ELISA plates coated with blood platelets followed by a washing step. vWF that remains bound to the blood platelets are detected by anti-vWF HRP labeled polyclonal antibodies with a Tecan Sunrise ELISA reader. In the absence of neutralizing ADA, vWF binding to blood platelets is blocked by the DP, and there would be minimal signal. In the presence of neutralizing ADA, the NAbs inhibit the DP from blocking the vWF-platelet interaction, resulting in increased signal. Titer calculation is based on linear regression of the dilution factor corresponding to the response values directly below and equal to or above the titration cut-point. The titration series is performed in steps of 2-fold dilution until negative scoring is achieved.

Essential Reagents and Concentrations

Positive Controls (PC):

Table 3: Positive Control samples and assay sensitivity

Name	Type of Reagent	Animal Source	Sensitivity in healthy patient serum (LOD)(ng/mL)
(PC1) pAb anti-DP rabbit pool (consisting of Rb24966 & Rb24967) (Used in Quality Control samples and likely to contain both neutralizing and non-neutralizing antibodies)	Polyclonal pool	Rabbit	155.2 and 71.3 (95% and 50% Confidence Interval (CI))
(PC2) mAb neutralizing anti-DP ABH0066	Monoclonal	Mouse	166 ng/mL (50% CI)
(PC3) pAb anti-DP Rb6869 (Also used in first validation report)	Polyclonal	Rabbit	84.6 (50% CI)

Sensitivity in serum from TTP patients: Using PC1: 91.7 ng/mL (50% CI)

Low Positive Control (LPC): 695 ng/mL (Based on 99% CI, referred to as LoQC2)

500 ng/mL (Referred to as LoQC3)

261.6 ng/mL (Based on 99.9% CI, referred to as LoQC1)

LoQC1 and LoQC2 calculated based on 6 LOD determinations using PC1. However, because LoQC2 is close to the plateau level of the LOD curve, a lower concentration, LoQC3, was proposed.

High Positive Control (HPC):	5,000 ng/mL
Negative Control (NC):	Pooled human serum (PHuS) Batch # PHuS#13SEP2016_1
Additional sera:	Healthy individual and pooled human serum, individual and pooled TTP patient human serum.
Reaction Mixture:	ALX-0081 (Batch ALX-0081-RS-04), ristocetin A sulphate, vWF, detection anti-vWF HRP labeled polyclonal rabbit antibodies
Minimal Required Dilution (MRD)	1/2.3 The MRD was established during method development and was not repeated during assay method validation.

Screening and Titration Cut Point Determination

The screening and titration cut-points were determined in parallel by running screening and titration assays simultaneously on the same plate using a balanced experimental design. The cut-point determination in serum samples from healthy adults was based on data from 292 samples from 50 subjects generated from 6 runs by 2 different operators over 3 days. The Applicant chose to determine **floating** screening and titration cut points.

To determine the cut points, the Applicant first determined the distributions of the blank population normalized by subtraction of the mean NC, division by the mean NC, or log transformation of the sample signal divided by the mean NC. The results indicated that distribution of the non-log transformed sample signal divided by the mean NC was normally distributed (Shapiro Wilk p-value = 0.27) and had the smallest skewness coefficient (0.114) and was therefore chosen for cut point determination.

Outlier exclusion was based on an iterative process using a mixed-effects model and Tukey's outlier criterion (OD <Q1 – 1.5IQR or >Q3+1.5*IQR where Q1 and Q3 are defined as the ODs at which 25% of the population had lower or higher ODs, respectively IQR is defined as Q3-Q1). Outlier exclusion was performed over multiple rounds that removed 13 analytical outliers and 1 biological outlier. In total, 275 samples were retained for cut-point determination.

Because the data distribution is normally distributed, the cut-points were determined using a parametric method:

Mean + 2.326*SD (1% false positive rate), Mean + 3.090*SD (0.1% false positive rate)

The results are summarized below:

Samples	Screening cut-point normalization factor (NF)	Titration cut-point NF
Healthy Adults	1.381	1.557

Reviewer's Comments:

The experimental design and statistical analysis used for cut point calculation is acceptable.

Assay Precision

Quality control (QC) samples consisting of healthy human sera spiked with LoQC1, LoQC2, and HiQC concentrations were prepared using PC1. To evaluate the precision of the QC and NC samples, 18 batches were evaluated by 2 analysts. The %CV for intra-run precision was <11% for all QC and NC samples. The %CV for inter-batch precision was <18% for all QC and NC samples.

All HiQC, LoQC1, and LoQC2 samples were screened positive and all NC samples screened negative. From these data, the Applicant concluded that the assay sensitivity is LoQC1 (261.6 ng/mL).

Reviewer's Comments:

The assay precision is acceptable.

Assay Suitability Control

The in-study acceptance criteria were established using data from all precision runs. The lower and upper limits for the QC samples are based on a 0.5% failure rate while the upper limit for the NC is based on a 1% failure rate using Student's t-distribution as summarized below (from the submission):

Lower limit normalized QC		Normalized QC value		Upper limit normalized QC
2.280	≤	HiQC	≤	**4.318**
2.044	≤	LoQC3	≤	**4.162**
1.436	≤	LoQC1	≤	**3.987**

		NC (Response)	≤	Upper limit NC (Response)
		NC	≤	**0.495**

Reviewer's Comments:

The approach to establish the in-study acceptance criteria for reagents is acceptable.

Assay Sensitivity

The assay sensitivity (Table 3) was verified for each of the different positive controls diluted in healthy human serum. For PC1, 6 independent batches were evaluated whereas for the other positive control samples, 1 batch was evaluated. Additionally, for PC1, sensitivity was determined in aTTP serum and in healthy adolescents as reported above. The sensitivities were determined by interpolating the concentration of the positive control antibody corresponding to the screening cut-point by fitting the concentration response curve using a linear regression.

Reviewer's Comments:

The sensitivity of the NAb assay is <100 ng/mL (50% CI) using PC1 in healthy adult, and in TTP patient sera. The approach used to determine assay sensitivity is appropriate.

Drug Tolerance

The Applicant evaluated the ability of the assay to detect NAb (PC1, PC2) in the presence of circulating drug and established drug tolerance limits for the assay. The result is provided in the following Tables from the submission:

PC1:

	(Interpolated) sensitivity at different concentrations drug			
	Concentration drug ALX-0081(ng/mL)			
	1000.0	100.0	10.0	0.0
(Interpolated) sensitivity (ng/mL)	<100.0 ng/mL	160.0 ng/mL	233.3 ng/mL	<100.0 ng/mL

PC2:

	(Interpolated) sensitivity at different concentrations drug			
	Concentration drug ALX-0081(ng/mL)			
	1000.0	100.0	10.0	0.0
(Interpolated) sensitivity (ng/mL)	<100.0 ng/mL	138.8 ng/mL	<100.0 ng/mL	<100.0 ng/mL

Note that in the presence of the highest amount of drug, the condition where no positive control was added (NC) also scored positive due to elevated baseline signals. Furthermore, in the presence of 1000 ng/mL DP, the OD signals are considerably reduced at all concentrations. Therefore, the Applicant concludes that there is no clear evidence that this assay allows sensitive detection of NAb in the presence of 1000 ng/mL drug.

Reviewer's Comment:

Overall, the assay sensitivity results indicate that the assay has low drug tolerance, and therefore the development of an alternative assay was appropriate.

Titer Precision

Titer calculation is based on linear regression of the dilution factor corresponding to the response values directly below and equal to or above the titer cut-point. Titer precision and MSR was determined for healthy adult sera (13.1%, 2.76) and TTP patient sera (12.8%, 2.83).

Reviewer's Comments:

The approach used to determine the precision of the titer assay is appropriate.

Stability

Stability of quality control samples with and without Protein G pre-treatment/complement inactivation was evaluated after freeze/thaw cycles, and real time storage at ultrafreezer, freezer, refrigerator, and incubator temperatures. All samples tested had >80% recovery and <20% intra-run precision except for pretreated samples (protein G eluates) at 1 week in the refrigerator and 2 weeks in the ultrafreezer. The results indicate that the quality control samples are stable under the studied conditions but that the eluates cannot be stored in the refrigerator or the ultrafreezer for more than 1 or 2 weeks respectively.

Reviewer's Comment:

The quality control samples are stable under the studied conditions.

Robustness

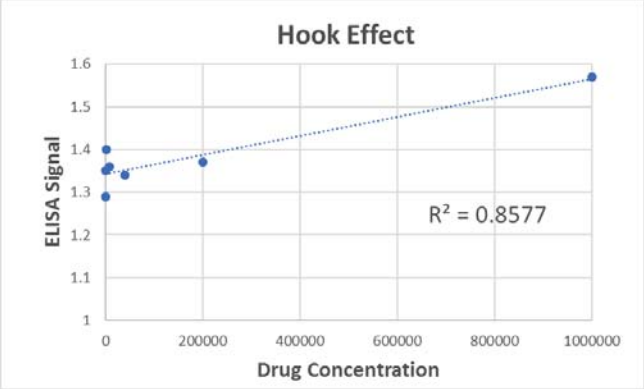
Robustness of the assay was evaluated by assessing inter-operator variability (2 different operators), assay drift (intra-plate drift) and influence of incubation time 0 (Assays with quality control samples performed with ±10% incubations times and ±5% overnight coating time). The results indicate that assay variability under these tested conditions did not affect the integrity of the data.

Reviewer's Comment:

The robustness of the assay is acceptable.

Hook Effect

To assess whether there is a hook effect, the Applicant measured the ELISA signal from the serial dilutions of PC1 beginning at 1 mg/mL (Table 26 of Validation Report AMV0044). The data is plotted below by the reviewer:



Reviewer's Comment:

The hook effect was not observed for this assay using PC1.

Selectivity – Matrix Interference and Target Interference

Matrix Interference of the assay was assessed by spiking PC1 at HiQC, LoQC1, and LoQC3 into hemolytic pooled human samples (2% hemolyzed whole blood spiked into blank pooled human serum) and lipemic pooled human samples (5 mg/mL triglycerides spiked into blank pooled human serum), which are then analyzed by the assay. For all samples, the in-study acceptance criteria and intra-run %CV were met. Target interference of the assay was assessed by spiking a serial dilution of vWF beginning at 50,000 ng/mL (100% serum level) into negative samples. During the first experiment, a false positive signal was detected from an interpolated vWF concentration of 4,815 ng/mL onwards. However, the first experiment study samples had not undergone complement inactivation. Complement inactivation was performed for the second experiment, for which a false positive signal was detected from an interpolated vWF concentration of 30,111.1 ng/mL onwards. The Applicant notes that the commercial vWF may not be fully representative of the native vWF present in patients, and that the presence of false positives due to elevated target levels will be assessed by correlating the measured target levels with NAb reactivity.

Reviewer's Comment:

Selectivity was appropriately evaluated.

Alternative Neutralizing Antibody Assay:

Method

The alternative NAb screening assay is an ECL based binding assay with the same bridging format as the ECL ADA binding assay described above. Briefly, human plasma samples are incubated with a master mix containing biotinylated and SULFO-tagged caplacizumab (1:1 at 1 µg/mL each) to form immune complexes consisting of ADA bound to the labeled caplacizumab molecules. Additionally, a null variant of the DP with altered CDR loops is included in excess (200 µg/mL) in the master mix. The Applicant claims that non-neutralizing antibodies would predominantly bind to the null DP while neutralizing antibodies will form immune complexes with the labeled DP. The labeled complexes are captured via the biotinylated caplacizumab on a MSD GOLD 96-well Streptavidin SECTOR plate and quantified by detection of the ECL signal from the SULFO-tag using a MESO QuickPlex SQ120 instrument. The titration assay uses the same ECL based bridging format. The assays do not incorporate any procedure to enhance sensitivity or specificity, such as acid dissociation or sample purification.

Reviewer's Comment:

In the initial submission, the Applicant did not provide scientific justification to support the following claims that are critical to the suitability of the alternative NAb assay:

1.) the null variant can bind to most non-neutralizing ADA, and therefore reduce the ECL signal from non-neutralizing ADA,

2.) the null variant has reduced binding to most neutralizing ADA, and therefore maintain the ECL signal from neutralizing ADA.

In response to an IR sent on 11/8/2018, the Applicant provided data and scientific justification in support of the suitability of the null variant for the alternative NAb assay (eCTD 0021) as summarized below:

During development of the alternative NAb assay, the Applicant first established a panel of neutralizing and non-neutralizing monoclonal antibodies (ABH####) and polyclonal antibody by screening for neutralizing activity as summarized in Table 4 of study report a068-c-2017-002 (Section 5.3.1.4 of the submission) as shown below:

Table 4: Overview of the neutralizing potential of positive controls in the 3 NAb assay set-ups

	Neutralizing activity	NAb assay set-up 1	NAb assay set-up 2	NAb assay set-up 3
ABH0013	neutralizing	+	+	+
ABH0054	non-neutralizing	-	-	-
ABH0058	non-neutralizing	-	ND	ND
ABH0066	neutralizing	+	+	+
Affinity purified pAb	neutralizing	+	+	ND
ABH0011	semi-neutralizing	±	-	±
ABH0071	non-neutralizing	-	-	-
ABH0070	non-neutralizing	-	-	-
ABH0016	non-neutralizing	-	ND	-

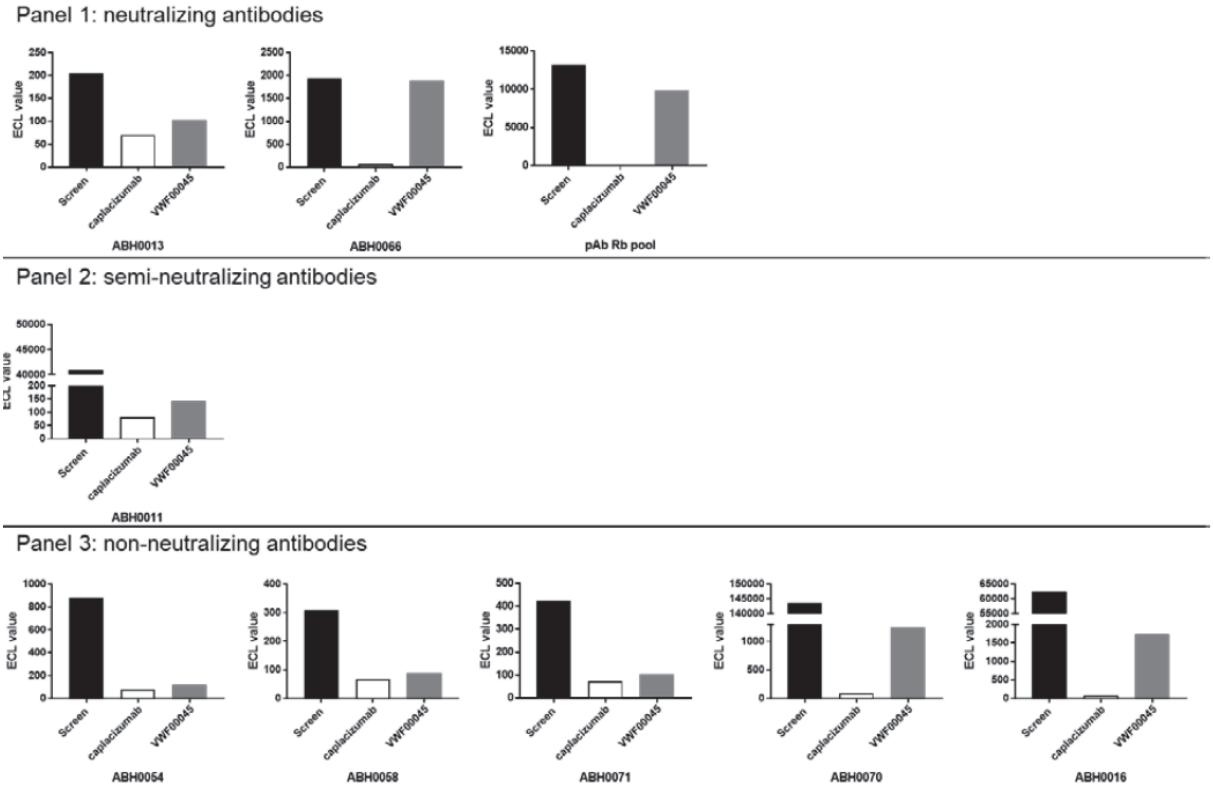
Note: NAb assay set-ups are format variations of the functional NAb assay.

The monovalent null variant was generated through iterative rounds of mutations to the CDR loops of the monovalent DP that eliminated binding to vWF and to the neutralizing antibodies while retaining binding to the non-neutralizing antibodies listed in the table above. The selected monovalent null variant, VWF0031 (the divalent null variant is VWF00045), showed lack of binding to vWF and ABH066 but did not lose binding to NAb ABH0013. Compared to the unmodified DP, the null variant had comparable affinity towards all non-neutralizing antibodies. The Sponsor also compared the crystal structures of the monovalent null variant to the monovalent DP.

Reviewer's Comment: *The crystal structures of the monovalent null variant and the monovalent DP differ primarily in the N-terminus, the CDR2 loop and surrounding framework residues, and the CDR3 loop; the rest of the framework regions are structurally comparable (refer to Figure 10 of study report a068-c-2017-002). However, these minor differences could be attributed to differences in crystal packing interactions. Overall, the structures suggest that ADA epitopes outside of the CDR loops are largely intact.*

The suitability of the null variant was further evaluated at 100 µg/mL with the panel of antibodies at 20 µg/mL in the table above in the alternative NAb assay as shown in Figure 11 of the study report a068-c-2017-002 from the submission and reproduced below. Note that the alternative assays performed in the presence of the null variant is compared to the results performed in the presence of unmodified DP at the same 100 µg/mL concentration.

Figure 11: Effect of an excess of the bivalent null variant VWF00045 (at 100 µg/mL) on detection of neutralizing and semi-and non-neutralizing antibodies (at 20 µg/mL) in the alternative NAb assay, compared to the ADA screening response and the confirmatory response with the bivalent caplacizumab (at 100 µg/mL)



Briefly, the results indicate that in the presence of excess DP, the alternative NAb assay fails to detect NAb ABH066 and the neutralizing pAb Rb pool. Furthermore, detection of ABH0013 is greatly reduced. In contrast, when the assays were performed in the presence of the null variant, ABH066 and pAb Rb pool were readily detected. However, detection of ABH0013 was comparable in the presence of DP and the null variant. In the presence of excess DP, non-neutralizing antibodies were not detected by the alternative NAb assay. This was also largely true for assays performed in the presence of excess null variant except for high affinity non-neutralizing antibodies ABH0070 and ABH0016. Subsequently, the Applicant observed that at a higher concentration of the null variant, 200 µg/mL, the ECL signal from the high affinity non-neutralizing ADA were reduced. Additionally, the assay signals and sensitivities obtained from the original ADA binding assay using several of the antibodies listed in the table above were compared to the alternative NAb assay in the presence of 200 µg/mL null variant. Both assays detected neutralizing antibody ABH0066 and pAB Rb pool similarly. The conventional ADA assay was substantially more sensitive when detecting the non-neutralizing and semi-neutralizing antibodies, which would be depleted in the presence of the null variant in the alternative NAb assay.

Finally, a set of 28 human serum samples, comprising both pre-Ab positive and negative samples, was analyzed in the alternative NAb assay as shown in Table 8 of the study report a068-c-2017-002 from the submission and reproduced below. The Applicant assumed that pre-Abs are non-neutralizing since they are not detected by the functional neutralizing assay.

Table 8: Optimization of the concentration of null variant of caplacizumab in the alternative NAb assay using pre-Ab positive individuals

	Screening	Confirmation with caplacizumab (100 µg/mL)		Null variant (100 µg/mL)	
(b) (6)	Average ECL	Average ECL	%Reduction	Average ECL	%Reduction
Ind 1 -	**218.0**	68.5	69%	77.0	65%
Ind 2 -	90.0	68.0	24%	85.5	5%
Ind 3 -	83.5	68.0	19%	76.5	8%
Ind 4 -	**150.5**	68.0	55%	93.5	38%
Ind 5 -	**769.5**	66.0	91%	79.5	90%
Ind 6 -	**971.5**	69.0	93%	77.0	92%
Ind 7 -	**192.5**	66.5	65%	84.0	56%
Ind 8 -	**1813.5**	73.0	96%	78.0	96%
Ind 9 -	106.0	79.5	25%	88.5	17%
Ind 10 -	84.5	64.0	24%	76.5	9%
Ind 11 -	81.0	66.0	19%	75.0	7%
Ind 12 -	132.0	65.5	50%	80.0	39%
Ind 13 -	**21880.5**	95.5	100%	100.0	100%
Ind 14 -	109.0	64.5	41%	74.5	32%
Ind 15 -	83.5	66.5	20%	82.5	1%
Ind 16 -	78.5	66.0	16%	78.5	0%
Ind 17 -	90.5	66.0	27%	76.5	15%
Ind 18 -	91.0	69.0	24%	75.5	17%
Ind 19 -	**393.0**	65.5	83%	75.0	81%
Ind 20 -	80.0	70.5	12%	75.0	6%
Ind 21 -	90.5	65.0	28%	85.5	6%
Ind 22 -	**301.5**	65.0	78%	78.0	74%
Ind 23 -	112.0	66.0	41%	84.0	25%
Ind 24 -	**6513.5**	68.5	99%	91.5	99%
Ind 25 -	76.0	62.5	18%	74.0	3%
Ind 26 -	83.5	64.0	23%	70.5	16%
Ind 27 -	81.5	62.5	23%	73.0	10%
Ind 28 -	**145.5**	64.0	56%	73.5	49%

Note: Screening results represented in bold screened positive

The reviewer calculated the following statistics from the data presented above:

Average ECL reduction of pre-Ab negative sample with null variant: 12.7% ± 2.6% (1 x S.E.M.*)
Average ECL reduction of pre-Ab negative sample with DP: 25.5% ± 2.4%
Average ECL reduction of pre-Ab positive sample with null variant: 76.4% ± 6.5%
Average ECL reduction of pre-Ab positive sample with DP: 80.4% ± 5.1%
****S.E.M. = Standard Error of the Mean***

The average ECL reduction is greater with excess of DP than excess of null variant, suggesting that the null variant lacks some of the epitopes that are recognized by non-neutralizing ADA. However, based on the Wilcoxon signed-rank test (distributions are not normal, calculations done using JMP), the difference in ECL reduction in the presence of excess null variant is not significantly less than in the presence of excess DP (p = 0.72). The results suggest that the differences in non-neutralizing ADA epitope exposure in the null variant and the DP may not be significant.

Reviewer's Comment:

Overall, the data provided by the Sponsor are supportive of the comparability of the null variant and the DP to reduce the ECL signal from non-neutralizing antibodies in human samples. However, the data indicates that the null variant can still bind to the neutralizing antibody ABH0013, suggesting that not all neutralizing antibodies would be detected by the alternative NAb assay. Nonetheless, the risk of neutralizing ADA is low because all subjects are concomitantly treated with immune suppressors and because neutralizing ADA does not appear to impact clinical efficacy in available clinical trial data. Therefore, the approach used in the alternative NAb assay is acceptable.

Because the validation strategy of the alternative NAb assay and the functional NAb assay are nearly identical including the positive controls, please refer to the review of validation of the functional NAb assay on the validation approach. Note that the same healthy adult serum samples and TTP patient serum samples used to validate the conventional ADA binding assay was used to validate the alternative NAb assay. For the cut-point determination, the same parametric formula was applied because the normalized, non-log transformed, outlier removed dataset (24 analytical outliers, 5 biological outliers) was normally distributed. Furthermore, the Sponsor refers to the validation of the conventional ADA binding assay for information regarding the stability of quality control samples. The validation results for the alternative NAb assay are summarized below in a reviewer generated table. The validation results for functional NAb assay results are included to aid in direct comparison:

	Functional NAb	**Alternative NAb**
Screening Cut-Point	Normalization Factor (NF) = 1.381 (1% FP)	NC= 1.262 (1% FP)
Titer Cut-Point	NF = 1.557 (0.1% FP)	NF= 1.321 (0.1% FP)
Quality Controls	HiQC: 5,000 ng/mL LoQC3: 500 ng/mL LoQC1: 261.6 ng/mL	HiQC: 20,000 ng/mL LoQC2: 443.7 ng/mL LoQC1: 188 ng/mL
Sensitivity	71.3 ng/mL (healthy human sera), 91.7 ng/mL (TTP patient sera)	60 ng/mL (healthy human sera), 28.8 ng/mL (TTP patient sera)
Intra-run precision	HiQC: 4.8% LoQC3: 6.2% LoQC1: 10% NC: 10% (AC: (b) (4) %)	HiQC: 4.5% LoQC2: 3.1% LoQC1: 6.4% NC: 3.3% (AC: (b) (4) %)

	Functional NAb	Alternative NAb
Inter-batch precision	HiQC: 11.5% LoQC3: 12.7% LoQC1: 17.5% (AC: (b) (4) %)	HiQC: 1.9% LoQC2: 11% LoQC1: 8.5% (AC: (b) (4) %)
Drug tolerance	233.3 ng/mL sensitivity in presence of 10 ng/mL DP,	≤ 100 ng/mL sensitivity in presence of 10 µg/mL to 1 µg/mL drug
Minimum Significant Ratio	Healthy patient sera: 2.76 (9.4% precision) TTP patient sera: 2.83 (11.8% precision)	Healthy patient sera: 2.13 (3.8% precision) TTP patient sera: 2.57 (4.8% precision)
Minimum Required Dilution	1/2.3 dilution	1/96 dilution

FP: False positive rate as established by a t-distribution, AC: Acceptance Criteria

Reviewer's Comment:

Overall, the validation results indicate that the alternative NAb assay is a more sensitive and drug tolerant assay than the functional NAb assay.

Jacek Cieslak

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Leopold Kong

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Leslie Rivera-Rosado

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Christopher Downey

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OFFICE OF DEVICE EVALUATION

DIVISION OF ANESTHESIOLOGY, GENERAL HOSPITAL, RESPIRATORY, INFECTION CONTROL, AND DENTAL DEVICES

GENERAL HOSPITAL DEVICES BRANCH

INTERCENTER CONSULT MEMORANDUM

Date	December 6, 2018
To	Melinda Bauerlien, RHPM CDER/OPQ/OPRO
Requesting Center/Office	CDER/OPQ/OPRO
OND Review Division	CDER/OHOP/DHP
From	Florencia Wilson CDRH/ODE/DAGRID/GHDB
Through (Team Lead)	Carolyn Dorgan CAPT Alan Stevens CDRH/ODE/DAGRID/GHDB
Through (Branch Chief)	CAPT Alan Stevens CDRH/ODE/DAGRID/GHDB
Subject	Consult for BLA 761112 ICCR2018-03339 ICC1800661
Recommendation	Recommendation Date: CDRH was not consulted.
Recommendation	Recommendation Date: 9/4/18 • CDRH is recommending that the device constituent of the combination product is not approvable for the proposed indication based on the lack of Essential Performance Requirements for the PFS on the final finished to be marketed combination product. • CDRH has Information Requests related to the device constituent parts of the combination product - See Section 11.3 for Information Requests
Recommendation	*Recommendation Date:12/6/18* *Device Constituent Parts of the Combination Product are Approvable with one (1) Post-Market Commitment, Section 13.2*

Digital Signature Concurrence Table	
Reviewer	Florencia T. Wilson -S Digitally signed by Florencia T. Wilson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011906324, cn=Florencia T. Wilson -S Date: 2018.12.06 12:09:43 -05'00'
Team Lead	Carolyn C. Dorgan -S Digitally signed by Carolyn C. Dorgan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001800814, cn=Carolyn C. Dorgan -S Date: 2018.12.06 16:12:38 -05'00'

Branch Chief	

1. Submission Overview

Table 1. Submission Information	
ICCR # (Lead)	ICCR2018-03340
ICCR SharePoint Link	http://sharepoint.fda.gov/orgs/OSMP/ocp/ICRR/Lists/ICRR%20Forms/Item/displayifs.aspx?List=337aa2e9%2D7692%2D4a76%2Dada9%2Dae967ad4a69b&ID=3657&Web=703664f2%2D33ef%2D4c9f%2Da6f2%2De1f658e187f9
ICC tracking # (Lead)	ICC1800661
Submission Number	BLA 761112
Sponsor	Ablynx
Drug/Biologic	Caplacizumab
Indications for Use	(b) (4)
Device Constituent	PFS and vial
Related Files	ICCR2018-03340 (CDRH-OC consult associated with this consult), IND 107609 (3/2016)

NOTE: All elements of this memo that are directly taken from the submission are in *bold / italics*.

Table 2. Review Team				
Were other disciplines consulted?			**☒ Yes**	**☐ No**
Below is a list of the Discipline Specific ICCR#, ICC# and CON#.				
Discipline Specific Consults	**Reviewer Name (Center/Office/Division/Branch)**	**ICCR #**	**ICC #**	**CON #**
CDRH-OC	M. Isabel Tejero del Rio, MD PhD	ICCR2018-03340	ICC1800661	CON1819745

Table 3. Important Dates	
Final Lead Device Review Memo Due	12/6/18
Interim Due Dates	**Due Date**
Filing	Finalized on 8/1/18 **[CDRH was not consulted prior to the filing review]**
74-Day Letter	CDRH was not consulted prior to the filing review
Mid-Cycle	8/29/18 (CMC/OPQ), 9/4/18 (CDER/OND)
Primary / Secondary Review	10/26/18 – CDRH due to CMC (OPQ) 11/6/18 – OPQ reviews due to OND
PDUFA/GDUFA Due Date	2/6/19

TABLE OF CONTENTS

Update TOC

2. PURPOSE/BACKGROUND

2.1. Scope

Ablynx is requesting approval of the caplacizumab. The device constituent of the combination product is a PFS. The PFS and drug/biologics are co-packaged in a convenience kit consisting of a vial adapter, a needle with safety shield, and two alcohol swabs.

CDER/OPQ has requested the following consult for review of the device constituent of the combination product on 08/13/2018:

Ablynx submitted biologic license application, 351(a)BLA) 761112, on June 6, 2018. OPQ is requesting a consult review of the pre-filled syringe with diluent to determine if the information provided is adequate to support approval of the BLA. Link to submission: \\CDSESUB1\evsprod\BLA761112\0003.

For CDRH OC (ICCR ICCR2018-03340): Ablynx submitted biologic license application, 351(a)BLA 761112, on June 6, 2018. Assess all relevant documentation in BLA 761112 regarding the pre-filled syringe (PFS) with diluent to determine if the information provided is complete and in compliance. Determine if an inspection is needed for the relevant facilities regarding the PFS including the sponsor of the BLA and/or the manufacturer of the PFS. If an inspection(s) is required, to perform the required inspection(s). Link to submission: \\CDSESUB1\evsprod\BLA761112\0003

The facility which manufactures the PFS with diluent is (b) (4)

The goal of this memo is to provide a recommendation of the approvability of the device constituent of the combination product. This review will cover the following review areas:

- Device performance
- Biocompatibility of the patient contacting components
- Release Specifications for the device constituent
- Sterility of the device constituent if applicable

A consult was also place in CTS to determine if an inspection is needed for relevant facilities regarding the PFS. The consult number is CON1819745.

Based on the e-mail received from CDRH-OC (M. Isabel Tejero, MD, Ph.D.) on 8/28/18:

Based on the tier chart we are currently using for the OPEQ pilot, CDRH does not need to conduct a compliance evaluation of the application.

This assessment pertains exclusively to the Compliance Review:

- Desk review of 21 CFR 820 call-outs, and
- Evaluation of manufacturing facilities to determine the need for inspections associated with this application.

The decision for not conducting a device compliance review for this application is independent from the technical review of the device constituent part done by our pre-market colleagues.

The Consult e-mail response from CDRH-OC is appended in Section 14, Appendix.

This review will not cover the following review areas:

- Compatibility of the drug with the device materials
- Human Factors

The original review division will be responsible for the decision regarding the overall safety and effectiveness for approvability of the combination product.

2.2. Prior Interactions

An ICC consult for IND 107609 was done by CDRH on 4/5/2016.

2.2.1. Related Files

- *IND 107609*

2.3. Indications for Use

Table 1: Indications for Use

Combination Product	Indications for Use
Caplacizumab	An anti-von Willebrand factor Nanobody indicated for patients (b) (4) (b) (4)
PFS (with 1 mL sterile for water injection as diluent)	Initial container for the sWFI and the same syringe to deliver the drug product
Vial Adapter (b) (4)	Transfer and mixing of drugs contained in vials
Hypodermic Needle (b) (4) Hypodermic needle (b) (4)	(b) (4) Needle device is intended for use in the aspiration and injection of fluids for medical purposes. (b) (4) Needle is compatible for use with standard luer slip and luer lock syringes. Additionally, after withdrawal of the needle from the body, the attached needle safety shield can be manually activated to cover the needle immediately after use. to minimize risk of accidental needlestick.
Alcohol swab (b) (4) Pre-amendment device	effective scrubbing and cleansing before the administration of a parenteral injection

3. ADMINISTRATIVE

3.1. Documents Reviewed

Document Title	Location
Cover Letters	GSR Sequences 0000-0010/1.2. Regional
Draft Labeling	GSR Sequence 0009/1.14.1.3. Regional
IND 107609	GSR
(b) (4)	Syringe
	510(k) Summary
	510(k) Summary

4. DEVICE DESCRIPTION AND PERFORMANCE REQUIREMENTS

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Dose accuracy / extruded volume		(b) (4) mg
Injection Time		Full volume is extruded
Injection Site		**First day of Treatment**: Intravenous (prior to plasma exchange) and Subcutaneous (after completion of plasma exchange) **Subsequent treatment during plasma exchange**: daily 10 mg subcutaneous injection following plasma exchange **Treatment after the plasma exchange period**: daily 10 mg subcutaneous injection for 30 days. If the underlying immunological disease is not resolved, treatment should be extended beyond 30 days and be accompanied by optimization of immunosuppression.
Injection tissue and depth of injection		Intravenous and Subcutaneous (injection in the abdomen, around the navel area avoiding the 2-inch area surrounding the navel, consecutive injections should not be given in the same abdominal quadrant), Depth = inject the full length of the needle at 45 to 90 degree angle
Audible / visual feedback		Visual (full volume is extruded)
Cap Removal Force	X	
Activation Force	X	
Visibility of medication container		The PFS is glass
Needle Specifications • Length(s) • Gauge(s) • Connection type o ISO 11608-2:2012 o Prestaked		Inside the co-packaged convenience kit (b) (4): • ½” • 30G • luer
Type of Use (e.g. single use, disposable, reusable, other)		Single use
Intended user (e.g., self-administration, professional use, user characteristics and / or disease state that impact device use)		(b) (4)
Method of actuation	X	
Automated Functions	X	
Residual Medication	X	
Drug Container Type		PFS
Dose Units of Measure (e.g., mL, Units, mg, increments, etc.)		mg/mL
Environments of use		Healthcare facility (hospital (Emergency Room, Intensive care Unit, hematology/nephrology department, outpatient clinic, general practitioner) and home
Storage conditions and expiry		refrigerator between 2°C to 8°C (36°F to 46°F), may also be stored at room temperature (up to 30°C or 86°F) for up to two months drug product store at 5°C ± 3°C, expiry period of 48 months

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6.3.2. Biocompatibility Review

Biocompatibility Evaluation		
Materials List	Plunger	Certificate of Conformity from (b) (4) in conformance to ISO 10993
	Syringe Body	Deferred to CDER, because the drug content/diluent is in contact with the inside of the syringe, hence it is applicable to the outside as well
	Needle	(b) (4) Hypodermic Needle is a 30G (b) (4)
	Accessories	Vial Adaptor 510(k) cleared device (b) (4)
Additives/Colorants		
Device Characteristics		
Category	☐ External communicating device	
Contact Type	☒ Blood path, indirect ☐ CSF contacting[1] [1]consult biocompatibility consultant	
Contact Duration	☒ ≤24h (limited) • Appropriate Endpoints: Cytotoxicity, Sensitization, Irritation, Acute systemic toxicity, Hemocompatibility (indirect hemolysis only), and Material-mediated Pyrogenicity ☐ >24h to 30 days (prolonged) • Appropriate Endpoints: Cytotoxicity, Sensitization, Irritation, Acute systemic toxicity, Hemocompatibility (indirect hemolysis only), Material-mediated Pyrogenicity, and Subchronic systemic toxicity ☐ >30 days (permanent) • Appropriate Endpoints: Cytotoxicity, Sensitization, Irritation, Acute systemic toxicity, Hemocompatibility (indirect hemolysis only), Material-mediated Pyrogenicity, Subchronic systemic toxicity, and Genotoxicity *Notes:* *The endpoints included in the biocompatibility evaluation of the needle are dependent on the type and duration of contact. Below are the recommended endpoints for blood path, indirect, which are applicable for both subcutaneous and intramuscular injections. If the needle is CSF contacting, information may be needed to support the evaluation of the neurotoxicity endpoint.* ***Repeated use of the device should be considered.*** *This includes cases in which a new syringe a is used each time the drug is administered. For example, if an injection given daily for 1 year would have a total exposure to the needle of ~10 seconds/day for 365 days which equates to ~1 hour.*	
Testing Performed	☐ Cytotoxicity ☐ Sensitization ☐ Irritation or Intracutaneous Reactivity ☐ Acute System Toxicity ☐ Material-Mediated Pyrogenicity *see review of Biocompatibility Testing	☐ Subacute/Subchronic Toxicity ☐ Genotoxicity ☐ Hemocompatibility ☐ Carcinogenicity

Did the Sponsor provide a written justification in lieu of biocompatibility testing	☐ **Yes**	☐ **No**	☒ **N/A**
IS the written justification acceptable?	☐ **Yes**	☐ **No**	☒ **N/A**

Review of Written Justification

Reviewer Comments/Conclusions

Did the Sponsor perform the appropriate testing?	☒ **Yes**	☐ **No**	☐ **N/A**

Review of Biocompatibility Testing

In review of the (b) (4) Biocompatibility, it was stated that the biocompatibility testing results of the testing demonstrate that the blood contacting materials are biocompatible.
The Sponsor also provided a conformity certificate from (b) (4) which was the supplier for the plunger rod, however, the texts are blurry. The Sponsor will be asked to provide the biocompatibility testing performed on the plunger rod per ISO 10993 and provide a clear/readable copy of the Conformity Certificate from (b) (4)

Additional comments:
The Sponsor provided a clear version of the Conformity Certificate located in 3.2.P.7 Solvent – Annex 10

This IR is resolved.

Reviewer Comments/Conclusions

Did the Sponsor complete a chemical characterization?	☐ **Yes**	☐ **No**	☒ **N/A**

Review of Chemical Characterization

Reviewer Comments/Conclusions

Biocompatibility Stock IR

Design Verification Recommendation		
The Sponsor Provided Complete Design Verification for the Device Constituent		☒
The Sponsor **DID NOT** Provide Complete Design Verification for the Device Constituent		☐
Design Verification Information Requests	Section 11.3 Mid-Cycle IRs - # Section 11.4 Interactive IRs - #	
All Information Requests were **Resolved** over the course of the review **[resolved on 11/27/18]**		☒
There are Complete Response Deficiencies, See Section 12		☐

7. RISK ANALYSIS

7.1. Risk Analysis Attributes

Risk Analysis Summary

Risk Analysis Attributes	**Yes**	**No**	**N/A**
Risk analysis conducted on the combination product	X		

Hazards adequately identified (e.g. FMEA, FTA, post-market data, etc.)	X		
Mitigations are adequate to reduce risk to health	X		

7.2. Summary of Risk Analysis

- In GSR Human Factor Engineering Report Reference DC-0036-v01 Pharmaceutical Development (Attachment 05) Sequence 0000/3.2.P.2. Quality, it referenced Risk Analysis (DC-0008) and Risk Management Report (DC-0035); however, the reviewer is not able to locate the documents in the BLA. Describe risk analysis process. An IR will be sent. Please see below.
- **This IR was resolved. Please see Section 11.**

Risk Analysis Recommendation		
The Sponsor provided complete Risk Analysis for the Device Constituent		☒
The Sponsor **DID NOT** provide There are Complete Response Deficiencies, See Section 13 Risk Analysis for the Device Constituent		☐
Risk Analysis Information Requests	Section 11.3 Mid-Cycle IRs - #1 – [**this IR was resolved on 9/24/18]** Section 11.4 Interactive IRs - #	
All Information Requests were **Resolved** over the course of the review **[resolved on 9/24/18]**		☒
There are Complete Response Deficiencies, See Section 12		☐

8. LABELING

Pre-Filled Syringe Labeling Checklist

Attribute		**Present**		
		Yes	**No**	**N/A**
Device Type (Cleared under (b) (4))	Type	X		
	Syringe Size(s)	X		
	Needle Gauge	X		
	Needle Length	X		
	Quantity	X		
Prescription Statement under 801.109(b)(1), except for insulin syringes		X		
Special requirements for insulin syringes as described in 801.403 about mixing insulin, including "For use with U100 insulin only" on the barrel and gradations on the barrel in units;				X
Any instructions for using specialized syringes such as the anti-needlestick devices and cartridge syringes;		X		
Any specific drug or biologic use;		X		
Instructions on how to clean and sterilize any reusable components.				X

8.1. Device Labels

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The Sponsor will perform the testing for resistance to overriding in accordance to ISO-80369-7 within six (6) months after approval and will be reported in an Annual Report.

13.Appendix

CDRH-OC Consult # CON1819745:

CABLIVI (caplacizumab) for injection (intravenous and subcutaneous use) is indicated for patients (b) (4) experiencing an episode of acquired thrombotic thrombocytopenic purpura (aTTP) (b) (4) This treatment is to be provided in conjunction with plasma exchange and immunosuppression. Dosage: (1) First day of treatment: 10 mg intravenous injection prior to plasma exchange followed by a 10 mg subcutaneous injection after completion of plasma exchange on that day. (2) Subsequent days of treatment during plasma exchange: daily 10 mg subcutaneous injection following plasma exchange. (3) Treatment after plasma exchange period: daily 10 mg subcutaneous injections for 30 days.

Based on the tier chart we are currently using for the OPEQ pilot, CDRH does not need to conduct a compliance evaluation of the application.

This assessment pertains exclusively to the Compliance Review:

- Desk review of 21 CFR 820 call-outs, and
- Evaluation of manufacturing facilities to determine the need for inspections associated with this application.

The decision for not conducting a device compliance review for this application is independent from the technical review of the device constituent part done by our pre-market colleagues.

Isabel

M. Isabel Tejero del Rio, MD PhD
Lead Consumer Safety Officer
OPEQ Pilot: OHT 3: Reproductive, Gastro-Renal, Urological, General Hospital Devices & Human Factors
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Excellent customer service is important to us. Please take a moment to provide feedback regarding the customer service you have received: Customer Service feedback

Melinda Bauerlien

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U.S. FOOD & DRUG ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Center for Drug Evaluation and Research
WO Building 22
10903 New Hampshire Ave.
Silver Spring, MD 20993

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date:	5 December 2018
To:	Administrative File, 761112/0
From:	Scott Nichols, Ph.D., Reviewer OPQ/OPF/DMA/BIV
Through:	Reyes Candau-Chacon, Ph.D., Quality Assessment Lead OPQ/OPF/DMA/BIV
Subject:	New 351(a) Biologics License Application (BLA)
US License Number:	2085
Applicant:	Ablynx NV
Manufacturing Site:	(b) (4)
Product:	Cablivi (caplacizumab, ALX0081)
Dosage:	sterile powder (10 mg) and solvent for solution for subcutaneous or intravenous injection
Indication:	(b) (4)
Action Date:	6 February 2018

Conclusion and Approvability Recommendation:

The drug substance portion of BLA 761112, as amended, is recommended for approval from a microbial control and a microbiological product quality perspective pending the submission of microbiology data to support in-process (b) (4)

The following post marketing commitment has been agreed to by the applicant:

To validate shipping of bulk drug substance from (b) (4) during summer conditions.

Please refer to the review memo from Dr. Candace Gomez-Broughton for an assessment of sterility assurance and microbiological product quality for the DP portions of the BLA.

Product Quality Microbiology Assessment: Drug Substance

Drug Substance Quality Microbiology Information Reviewed

Sequence number	Date	Description
0001	4 April 2018	Original BLA
0010	22 August 2018	Response to FDA Information Request (dated 7 August 2018)
0016	19 October 2018	Response to FDA Information Request (dated 11 October 2018)
0022	16 November 2018	Response to FDA Information Request (dated 6 November 2018)

Review Narrative, Module 3.2

S.1 General Information

ALX-0081 (caplacizumab) is a humanized, bivalent nanobody directed toward the A1 domain of human von Willebrand factor. (Nanobodies are derived from Camelidae family, which have unique immunoglobulins: These heavy-chain only antibodies contain a single domain immunoglobulin domain that is used for epitope recognition.) As ALX-0081 is a bivalent nanobody, it is composed of two identical, humanized variable chain domains that are joined together by a tri-alanine linker. The total protein size (27.9 kDa), and it is expressed in *E. coli* at commercial scale (~ 1 kL).

SATISFACTORY

S.2 Manufacture

(b) (4)

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Conclusion

I. The drug substance section of BLA STN 761112/0 was reviewed from a product quality microbiology perspective and is recommended for approval.

II. Product quality aspects other than microbiology should be reviewed by OBP.

III. Pre-license inspections of the (b) (4) manufacturing facilities were conducted from (b) (4) The facility was classified as NAI.

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Scott Nichols

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Reyes Candau-Chacon

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