11 November 2021
EMA/CHMP/631976/2021
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

HemAryo

International non-proprietary name: eptacog alfa (activated)

Procedure No. EMEA/H/C/005547/0000

Note

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

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Administrative information

Invented name of the medicinal product:	**HemAryo**
INN (or common name) of the active substance(s):	EPTACOG ALFA (ACTIVATED)
Applicant:	UGA Biopharma
Applied Indication(s):	HemAryo is indicated for the treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups: • in patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 Bethesda Units (BU) • in patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration • in patients with acquired haemophilia • in patients with congenital FVII deficiency • in patients with Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available.
Pharmaco-therapeutic group (ATC Code):	Blood coagulation factors (B02BD08)
Pharmaceutical form(s) and strength(s):	powder and solvent for solution for injection, 1,2 mg (60 KIU)

Declarations

This application includes an Active Substance Master File (ASMF):

☒ No

☒ The assessor confirms that this assessment does **not** include non-public information, including commercially confidential information (eg. ASMF, information shared by other competent authorities or organisations, reference to on-going assessments or development plans etc), irrespective from which entity was received.

List of abbreviations

Clinical

ADA	Anti-Drug Antibody
AE	Adverse event
ALT	Alanine aminotransferase
aPCC	Activated prothrombin complex concentrate
APTT	Activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the plasma concentration vs time Curve
AUC_{extra}	Fraction of the total AUCinf that was derived by extrapolation beyond tlast
AUC_{inf}	Area under the plasma activity-time curve from time 0 to infinity
AUC_{last}	Area under the plasma activity-time curve from time 0 to last quantifiable activity
BLOQ	Below the limit of quantification
BLQ	Below the Limit
BU	Bethesda Unit
CHMP	Committee for Medicinal Products for Human Use
C_{max}	Maximum drug concentration in plasma
CI	Confidence Intervals
Cl	Clearance
Cl_{sys}	Systemic Clearance
CSR	Clinical Study Report
CV	Coefficient of variation
CVp	Coefficient of variation of the PD endpoint of interest
EC	Ethical Committee
eCRF	electronic Case Report Form
EMA	European Medicines Agency
ETP	Endogenous Thrombin Potential
EU	European Union
F1.2	F1.2 prothrombin fragments
FVII	Coagulation Factor VII
FVII:C	FVII coagulation activity assay
FVIII	Coagulation Factor VIII
FIX	Coagulation Factor IX
FX	Coagulation Factor X
GCP	Good Clinical Practice
Hb	Haemoglobin
IM	Immunogenicity
IU	International Unit
kg	Kilogram
KIIa	Peak height divided by lag time of TGA measurements
L, dL, mL	Litre, decilitre, milliliter
LDH	Lactate dehydrogenase
LLOQ	Lower Limit of Quantification
MA	Marketing authorisation
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
min	Minutes
MRT	Mean Residence Time
MW	Molecular Weight
PAS	PK/PD Analysis Set
PCC	Protein Complex Concentrate
PD	Pharmacodynamic
PD_{marg}	PD margins
PI	Principal Investigator
PK	Pharmacokinetics
PT	Prothrombin Time
QC	Quality Control
QCH	High Quality Control
QCL	Low Quality Control

QCLL	Lower Quality Control
QCM	Medium Quality Control
rFVIIa	Recombinant FVII activated
RMP	Reference Medicinal Product
SA	Scientific Advice
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDS-PAGE	Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis
SES	Safety Evaluation Set
SEM	Standard Error of the mean
SmPc	Summary of Product Characteristics
SOC	System Organ Class
SOP	Standard Operating Procedure
STD	Standard Deviation
TF	Tissue Factor
TGA	Thrombin Generation Assay
t_{max}	Time to reach maximum drug concentration in the measured
$t_{1/2}$	Plasma half-life
ULN	Upper Limit of Normal
V_d	Volume of Distribution
Λz	First order rate constant associated with the terminal (log-linear) portion of the curve

1. CHMP Recommendations

Based on the review of the data on quality, pharmacology, safety, efficacy, the application for HemAryo in the treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups:

- patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 Bethesda Units (BU),
- patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration,
- patients with acquired haemophilia, patients with congenital FVII deficiency, and
- patients with Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available

is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions (see section VI).

In addition, satisfactory answers must be given to the "other concerns" as detailed in the List of Questions.

The major objections precluding a recommendation of marketing authorisation, pertain to the following principal deficiencies:

- The applicant did not sufficiently assess API's critical quality attributes, and thus the basis for the whole control strategy of the manufacturing process is missing
- Time-dependent variability, inter-roller bottle and inter-batch consistency have not been assessed, nor confirmed, and the current manufacturing process is not covered by pilot experiments. DS manufacturing process including harvest, pooling and cycling strategies was not comprehensibly described. It lacks critical information, precluding an assessment
- The methodological portfolio to control HemAryo DS is insufficient and fundamental methodological details to evaluate its applicability were not disclosed, precluding an assessment of the suitability of presented analytical portfolio to control DS's critical quality attributes
- The control strategy of the DS and DP manufacturing process is insufficient, and process performance is not assured. Submitted information about process validation does not fulfil the most basic principles of cGMP, thus validity of the DS and DP manufacturing process is not demonstrated
- Information on Reference Materials as provided by the applicant is incomplete, the Reference Standard seems unstable, and strategy to establish a new RS is not acceptable
- An affinity matrix produced by the applicant in an uncontrolled process by a non-characterised hybridoma in the presence of undocumented material of animal origin is part of the DSP and leads to a major safety and reproducibility risk
- Undocumented material of animal origin (FCS and trypsin) is routinely used to manufacture the API and to produce cell banks, and was used to establish the production cell line, which leads to a major safety risk
- A potential presence of nitrosamines in DS and DP was not assessed
- Insufficient CMC documentation relating to the solvent of HemAryo
- Major deficiencies in the design and conduct of the biosimilarity exercise
- Viral safety
- Results on *in vitro* PD are not considered to be sufficiently sensitive and discriminatory to detect potential differences between the biosimilar candidate and the comparator
- Methodological concerns impeding the similarity evaluation of the predefined primary PD parameters in the pivotal clinical study UGA 2014-01
- Failure to show equivalence in PD between HemAryo and NovoSeven in the pivotal clinical study UGA 2014-01
- Lack of justification for extrapolation to the indications not covered by the pivotal clinical studies
- Major deficiencies in the assay used for the immunogenicity assessment.

1.1. Questions to be posed to additional experts

None.

1.2. Inspection issues

1.2.1. GMP inspection

For the time being, a product-related GMP inspection would be worthless. First all CMC related MOs listed above need to become resolved, and their resolution might trigger adaptations of the current manufacturing process. After this, a request for GMP inspection is required for the following site(s) in order to verify the GMP compliance status and further product specific information.

The outcome of this/these inspection(s) is required for the Committee to complete its examination of the application and will be needed by Day 181.

1.2.2. GCP inspection

No GCP inspection is requested.

1.3. New active substance status

Not applicable for biosimilars.

1.4. Additional data exclusivity /Marketing protection

Not applicable for biosimilars.

1.5. Similarity with authorised orphan medicinal products

It is considered that HemAryo is not similar to Alprolix or Idelvion within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

2. Executive summary

2.1. About the product

The drug product HemAryo (drug substance eptacog alfa (activated), as per International Nonproprietary Names) is an activated recombinant human coagulation factor VII (rhFVIIa) concentrate which belongs to the pharmacotherapeutic group of Blood Coagulation Factors. HemAryo is a recombinant analogue of human FVIIa, a vitamin K-dependent coagulation factor. In the presence of both calcium and phospholipids, FVIIa in a complex with tissue factor (TF) activates factor X (FX) to factor Xa (FXa), directly bypassing the reactions that require factor VIII (FVIII) or factor IX (FIX). Activation of FX to FXa initiates the common pathway of the coagulation cascade in which prothrombin is activated to thrombin, which then converts fibrinogen to fibrin to form a haemostatic plug, thereby achieving clot formation at the site of haemorrhage (haemostasis). This process may also occur in the absence of tissue factor on the surface of activated platelets.

HemAryo is produced by recombinant deoxyribonucleic acid (DNA) technology in stable transfected BHK-570 cells. Cells are cultivated in roller bottles for 21 days while the secreted expression product is harvested daily from the culture supernatant. It is pooled and subsequently purified and activated

during the purification process to FVIIa. The glycoprotein produced (FVII) consists of 406 amino acid residues (molecular weight 50 KDa) which is structurally similar to human plasma derived coagulation FVIIa and has similar functional properties to human plasma-derived FVIIa and to another recombinant FVIIa (eptacog alfa).

HemAryo has been developed as a biosimilar to the reference product Novoseven. The applicant is claiming all of the approved indications of the reference product, namely treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups: patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 Bethesda Units (BU), patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration, patients with acquired haemophilia, patients with congenital FVII deficiency, and patients with Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available.

2.2. The development programme/compliance with guidance/scientific advice

Scientific advice and one follow-up advice were given to the applicant:

The first scientific advice from February 2015 (**EMA/CHMP/SAWP/168901/2015**) addressed following items:

Immunogenicity being of primary importance, shall be investigated with the final product to be marketed, and according to EMA-guideline "Immunogenicity assessment of biotechnology-derived therapeutic proteins" (EMEA/CHMP/BMWP/14327/2006). The company did not follow the scientific advice and did not adopt a testing strategy in line with the guideline listed above. The Assay presented by the Company is a potency assay, irrelevant for HemAryo's immunogenicity profile. It is independent from the DP and any contained immunogenic motif and does not assess its immunogenicity at all.

Concerning the quality the applicant did not fully follow the recommendations given in the advice. In particular, the applicant was advised to use a sufficient number of batches of the reference medicinal to establish similarity ranges. This recommendation was not incorporated into the development programme. In addition, it was outpointed that differences between AryoSeven and RMP should be further characterised and their impact evaluated. Also this issue was not addressed in the MAA. Finally, a more in-depth going biological characterisation was recommended; Unfortunately, this specific advice was not taken on board. In summary, these issues resulted in a Major Objection in the biosimilarity part.

With regard to the non-clinical development it was agreed with the applicant that *in vivo* studies will not be required for the biosimilar application. However, it was emphasised that a thorough *in vitro* functional characterisation of the biosimilar candidate in comparison with the reference product will be required. In particular it was pointed out that these studies should compare product at the pharmacological target(s), covering a concentration range where potential differences are most sensitively detected. Referring to the documents submitted in the briefing book it was further noted that clotting studies do not show data for zero concentration eptacog alfa and that in the descending part of the dose-effect curve no data points were presented. Moreover, the applicant was advised to discuss the relevance of the presented assays for haemophilia A/B patients with inhibitors. These recommendations were not considered by the applicant for the MAA.

A follow-up advice from August 2016 (**EMA/CHMP/SAWP/590140/2016**) addressed following items:

Again, the applicant was advised to assess Immunogenicity of HemAryo according to EMA-guideline "Immunogenicity assessment of biotechnology-derived therapeutic proteins" (EMEA/CHMP/BMWP/14327/2006). The applicant did not follow the scientific advice and did not adopt a testing strategy in line with the guideline listed above. The assay presented by the applicant is a potency assay, which is independent from the DP and any contained immunogenic motif, and does not assess its immunogenicity at all.

Scientific advice on clinical development:

Following the Scientific Advice received in 2015 and 2016, the applicant adopted the CHMP's recommendation and conducted two pivotal studies (UGA 2014-01 and UGA 2014-02) in support of a marketing authorisation of a biosimilar medicine. As recommended, the studies IRTC201202106302N2 and IRTC201104266302N1 were submitted as supporting studies.

The recommendations for study UGA 2014-01 (patients with haemophilia A/B with inhibitors) were largely adopted. However, the applicant did not provide any statistical or clinical justification for the PD margin used, which is of central importance to the assessment of the trial. The recommendations for study UGA 2014-02 were only partly adopted; the proposed immunogenicity group has not been conducted. An extrapolation to the indications not covered in the pivotal studies has not been discussed or justified.

2.3. General comments on compliance with GMP, GLP, GCP

GMP: The lyophilised drug product as well as the solvent (sterile water for injection) is manufactured and filled at the applicant`s manufacturing site Alborz in Iran. As the latest GMP inspection conducted by the Bulgarian Drug Agency had been conducted on 23/05/2018, the validity of the GMP certificate was automatically extended to the end of 2021 due to pandemic restrictions. This special measure will be further extended to the end of 2022 without the need of any action on the part of the certificate holder.

The applicant submitted a dossier of extremely poor quality. Many process and product details were missing, and important information was withheld by the applicant. MOs were raised for almost all sections of Module 3 and it appears that fundamental principles of current GMP were not fully understood and thus not followed. Severe doubts were raised about GMP status of HemAryo's manufacturing and control strategy.

For the time being, a GMP inspection would be worthless. First all CMC related MOs need to become resolved, and their resolution might trigger adaptations of the current manufacturing process. After resolution of CMC related MOs, a pre-approval inspection for human medicinal products is requested in accordance with Article 8(2) of Regulation (EC) No 726/2004 and Article 111(1) of Directive 2001/83/EC, which shall cover manufacture of active substance and manufacture of finished product, both carried out.

GLP: Not applicable as no safety, pharmacology or toxicology studies were conducted.

GCP: The two pivotal clinical studies were conducted in medical centres mostly outside the EU: Study UGA 2014-01 in centres in Iran, Turkey, Bulgaria and Georgia and study UGA 2014-02 in Iran and Turkey. The applicant should provide the outcome of the GCP inspections conducted at the two study sites in Iran. In addition, the applicant should clarify if the clinical study sites involved for any clinical trial have been inspected by an EU inspectorate, WHO or other competent authority. The outcome of any inspections should be provided. If there are no previous inspections, the applicant should present evidence that the studies has been conducted and analysed in accordance with international quality standards and requirements, for example via an audit report (**OC**).

2.4. Type of application and other comments on the submitted dossier

2.4.1. Legal basis

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC, as amended – relating to applications for biosimilar medicinal products.

2.4.2. PRIME

Not applicable.

2.4.3. Accelerated assessment

Not applicable.

2.4.4. Conditional marketing authorisation

Not applicable for biosimilars.

2.4.5. Marketing authorisation under exceptional circumstances

Not applicable for biosimilars.

2.4.6. Biosimilarity

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form:
    - NovoSeven, 1 mg (50 KIU) powder and solvent for solution for injection
    - NovoSeven, 2 mg (100 KIU) powder and solvent for solution for injection
    - NovoSeven, 5 mg (250 KIU) powder and solvent for solution for injection
    - NovoSeven, 8 mg (400 KIU) powder and solvent for solution for injection
- Marketing authorisation holder: Novo Nordisk A/S
- Date of authorisation: 23-02-1996
- Marketing authorisation granted by:

 Union

- Marketing authorisation number: EU/1/96/006/008, EU/1/96/006/009, EU/1/96/006/010, EU/1/96/006/011

2.4.7. New active substance status

Not applicable for biosimilars.

2.4.8. Orphan designation

Not applicable for biosimilars.

2.4.9. Similarity with orphan medicinal products

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report, addressing the possible similarity with authorised orphan medicinal products. Assessment of these claims is appended.

2.4.10. Information on paediatric requirements

Not applicable

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

The finished product HemAryo (active substance (AS) eptacog alfa (activated), as per INN) is an activated recombinant human coagulation Factor VII (FVIIa) concentrate which belongs to the pharmacotherapeutic group of Blood Coagulation Factors.

HemAryo is a recombinant analogue of human FVIIa produced by recombinant deoxyribonucleic acid (DNA) technology and secreted into the culture supernatant of stable transfected baby hamster kidney cells. In the presence of both calcium and phospholipids, the vitamin K-dependent coagulation factor FVIIa in a complex with tissue factor (TF) activates factor X (FX) to factor Xa (FXa), directly bypassing the reactions that require factor VIII (FVIII) or factor IX (FIX). Activation of FX to FXa initiates the common pathway of the coagulation cascade in which prothrombin is activated to thrombin, which then converts fibrinogen to fibrin to form a haemostatic plug, thereby achieving clot formation at the site of haemorrhage (haemostasis). This process may also occur in the absence of TF on the surface of activated platelets (Hoffman 1998).

HemAryo has been developed as a biosimilar to the reference product Novoseven. The applicant is claiming all of the approved indications of the reference product, namely treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups:

- patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 Bethesda Units (BU),
- patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration,
- patients with acquired haemophilia, patients with congenital FVII deficiency, and
- patients with Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available.

The finished product (FP) is presented as lyophilisate containing 1.2 mg of eptacog alfa (activated) as active substance. Other ingredients are: sodium chloride, calcium chloride dehydrate, glycylglycine, polysorbate 80 and D-mannitol. The product is supplied in rubber stopper sealed type 1 glass vials with flip-off aluminum caps. A second glass vial containing water for injections (WFI) for reconstitution of the finished product is contained within the secondary packaging.

3.1.2. Active Substance

3.1.2.1. General Information

The international nonproprietary name (INN) for recombinant recombinant blood-coagulation factor VII (activated), is eptacog alfa (activated).

Coagulation factor VIIa (Recombinant) (rhFVIIa) is a vitamin K-dependent glycoprotein consisting of 406 amino acid residues and has a molecular weight of 50 kilodaltons (kDa). During its purification, the recombinant DNA-derived factor VII protein is activated to the serine protease factor VIIa. Activation occurs by cleavage of the single chain molecule on the C-terminal side of the Arg152 residue, to produce an N-terminal derived light chain (LC) of 20 kDa (152 residues) and a C-terminal derived heavy chain (HC) of 30 kDa (254 residues), which remains covalently associated by a single disulfide bond.

Recombinant human FVIIa possesses a modular organisation with an N-terminal membrane-binding γ-carboxyglutamicacid (Gla)-containing domain, two epidermal growth factor (EGF)-like domains, and a C-terminal serine protease domain. Recombinant human FVIIa contains 12 disulfide bridges, seven of which are located on the light chain, four on the heavy chain, and one which enables the light and heavy chains to remain covalently bound once cleaved (interchain).

The active protein is glycosylated with two N-linked glycosylation sites (one on its LC at Asn145 and one on its HC at Asn322) and two O-linked glycosylation sites (on its LC, at Ser52 and Ser60) and the molecule gets ß-hydroxylated at Asp63 and contains γ-carboxylated glutamic acid residues which - together with multiple Ca^{2+} binding sites on the light chain and one on the heavy chain - are essential for functional activity.

3.1.2.2. Manufacture

Eptacog alfa activated (rFVIIa) is a 50 kDa glycoprotein produced by a genetically transformed Baby Hamster Kidney (BHK) cell line in a roller bottle production system and purified by consecutive purification steps.

Recombinant FVII is expressed as a single chain protein, which during purification gets activated by auto-proteolytic cleavage into the activated form denoted rFVIIa.

In general, the dossier was of extremely poor quality. The applicant provided CTD sections with very limited and short texts, without any discussion. Generally, the content of most of the active substance sections in module 3 was rather superficial and incomplete and often at the wrong place in the dossier. Besides mandatory activities which were not performed at all, specific information in many cases was missing, and thus the assessment of suitability of development activities often was impossible, resulting in several MOs.

The last GMP inspection to the active substance and finished product manufacturing site from a CEE authority dates from May 2018. Dating more than 3 years ago, a concern was raised about the GMP status of the AS and FP Manufacturer. Batch definition and nomenclature for the AS and FP were sufficiently explained. The batch size was described.

The applicant gave a high level overview of the active substance production process and provided a flow diagram.

A high-level description on Master Cell Bank (MCB), Working Cell Bank (WCB) and End of Production Cell bank (EoPC) preparation was presented. Information on storage locations and conditions, redundancy, cell line stability and retest-dates of cell banks is missing. No details on the expression host - cell line history (BHK cells) were given. Routine characterisation of MCB and WCB as presented in principle seems aligned with ICH Q5A (R1) and ICH Q5D guidelines, but the panel of viral safety assays needs to be enlarged and applied at WCB level. Presented analytical panel for the characterisation of the EoPC lacks for crucial attributes considering genetic stability and safety relevant tests, and the limit of *in vitro* cell age. The cloning strategy of rhFVIIa was sufficiently described.

Description of process controls consisted of listing some selected process parameters, without any link to the active substance CQAs or any explanation regarding their criticality. No quality risk assessment was performed. No difference was made between process input and output parameters, nor their criticality. Very broad acceptance criteria for some process parameters were listed, but not justified. Many process parameters which seem to be critical remain uncontrolled. Also testing strategy of bulk harvest and other intermediates was not included, nor explained.

Thus, the active substance manufacturing process was not sufficiently described and not discussed at all. In process controls and the way they were implemented in combination with unjustified process parameter ranges seem insufficient to control AS CQAs. No risk assessment was performed, and consequently no risk mitigation strategy was presented. A MO was raised due to the lack of information on the production process and an adequate control strategy.

Reagents, solvents and components used in the manufacturing process of HemAryo AS, their application and corresponding quality standards were listed. Besides compendial materials referenced to Ph. Eur. monographies, pharma-grade material, cell-culture grade material, in-house tested material and technical grade materials were used, without providing any further information on specified acceptance criteria. Thus, the applicant was asked to provide the full information about respective material specifications as well as information about release testing procedures and methods and their qualification. Material of filters was disclosed, but information regarding provenience and manufacturers of filters, certificates from the manufacturer, verification of certification and packaging was missing and was requested. Manufacturer qualification and material testing policy were disclosed and seems acceptable.

Material of animal origin is contained in growth media used both in each manufacturing campaign and in media for cell banking and cell preservation. No information on provenience of the material of animal origin, batch numbers, certificates or compliance statements was provided, and no risk assessment was performed. A MO was raised due to the severe safety risk of using undocumented materials of animal origin as raw material for the manufacturing of the API, and for cloning of the expression cell line, producing and storing cell banks of the production cell line.

No cell line history was provided. Cultivation conditions including media used for cell banking, batch numbers and certificates were not found. A MO was raised due to severe concerns due to a major safety risk by using undocumented materials of animal origin without any virus reduction steps for the manufacturing of a product which is in direct contact with the API.

The potential presence of nitrosamines in the AS in the FP was not evaluated, leading to another formal MO.

Variability amongst different rollers within one production campaign and the impact of the increasing cell age during the culture on CQAs of the API was not assessed. In contrast, data from a cell line stability study indicated a considerable drop of product titers already before the end of the second

week. Considerable differences were seen between the three experiments performed. Genetic stability of the cell line, intra-batch consistency and inter-batch consistency were not assessed. Such evidence should be supported by appropriate analysis of performance indicators and quality attributes which should be demonstrated to be consistent throughout all harvesting steps. Otherwise an approach to manage the variability of harvests should be proposed and validated. Cultivation conditions and product quality attributes were only assessed at highest level. These data do not support the current setting used for manufacture of HemAryo and therefore a further MO was raised.

The applicant evaluated operational parameters for several consecutive AS production campaigns and compared those parameters to predefined but not justified the acceptance ranges. Only a minor part of CPPs was evaluated, while their impact on process performance and product CQAs was not assessed. Many physico-chemical, safety and efficacy relevant input and output parameters were not monitored. Pre-defined acceptance criteria for validation parameters were met, but acceptance ranges were broad and not justified. Validation runs did not fulfil batch definitions and it is unclear if they can be considered as representative. Important details about the manufacturing process are not defined, nor controlled. Column lifetime studies are incomplete or missing for the most chromatography media. Validation of filtration steps was not addressed at all. The validity of the manufacturing process is not demonstrated and a MO was raised.

Development activities performed by the applicant ending up in the current version of the production process were described. API's biological activity was not taken into account. Optimised parameters eventually were verified in several pilot-scale production campaigns. Taken together, the output parameters monitored during these pilot experiments showed a considerable time and batch specific variability. Due to the lack of information and to major inconsistencies, it can not be deduced from pilot experiments that the process is capable of delivering rhFVIIa of constant and reproducible quality.

Several changes have been introduced to the manufacturing process during development. However, the changes have not been documented, evaluated or justified and no comparability studies for batches produced before and after the changes have been provided. The down scaled model applied to assess column lifetime was not representative. Taken together, development work of most manufacturing steps - ending up in the current version of the production process - was at least partially described.

3.1.2.3. Characterisation

Regarding the characterisation of HemAryo AS, again very limited information was available. The applicant gave a high-level overview of general properties of rhFVIIa which is mainly based on respective Ph. Eur. monography.

Characterisation has been performed by analysing several FP batches, which is unusual since normally AS is used for characterisation. The characterisation exercise presented in the corresponding section of the dossier is very incomplete. However, additional characterisation was performed in the context of the biosimilarity study, in which several batches of AryoSeven (FP) were compared to several batches of NovoSeven. A more complete panel of methods was applied for this study although many deficiencies were detected.

Methods used for deeper characterisation were listed by their names, but no information was given about methodological details, and their qualification to assess specific properties of the API. Only selected data were provided, without any explanation, interpretation or discussion. The methodological portfolio needs to be completed to become acceptable for assessment of physico-chemical properties and post-translational modifications of the API. On top of release assays, which were the ones described in the Ph. Eur. monography, some additional tests were performed. Only one assay was used

to study biological activity of rhFVII, which seems insufficient considering the whole spectrum of biological interaction partners of the molecule. Data were missing or quality was not acceptable: annotation and legends on chromatograms and gels were completely missing or unreadable, thus it was impossible to interpret respective data. rhFVII is a complex, highly post-translationally modified recombinant protein. Its complexity was only partially assessed. The correlation between post translational modifications, and their (potential) impact on pharmacology, safety and activity was not assessed. Biological function of the main product and variants only was poorly addressed. Product related impurities were not characterised, and their impact on activity, efficacy and safety was not analysed in order to address their criticality. Obvious heterogeneity of batches and a potential impact was also not addressed. Thus the API pattern of heterogeneity lacks important details. Process related impurities were only incompletely assessed. Chemical leachables and elemental impurities were not addressed. Assessment of adventitious agents was incomplete. Thus, characterisation of the API not only is incomplete but lacks essential information required to identify CQAs of the API, including but not restricted to structure, post-translational modifications, variants, degradation products and impurity profile. A MO was raised since this information is essentially required to understand the API's critical quality attributes, as basis for the manufacturing process control strategy.

3.1.2.4. Control of Drug Substance

The proposed active substance specifications were provided. Methods and acceptance criteria were indicated. The control of the extraordinary complexity of the molecule needs to be reflected in the analytical portfolio used to assess quality relevant properties of rhFVIIa.

The AS specification as proposed by the applicant mainly focuses on the methodological portfolio and acceptance criteria described in Ph. Eur. 2534. Considering the large spectrum of unidentified product variant forms and the deficiencies in the AS characterisation section, and regarding the extraordinary complexity of the protein rhFVIIa, further emphasis should be put on the analysis and the control of product micro-heterogeneity, to ensure batch to batch consistency and product stability. Based on the limited information on CQAs provided by the applicant, and the significant amount of unknown product related impurities, the quantity of the functional active main form should be controlled.

The applicant presented a brief summary only of the analytical portfolio used for rhFVIIa DS release and shelf-life. Besides very general aspects about applied methods, no information was given on methodological details like e.g. protocols, used reagents, buffers, media, columns, and how raw data are processed and evaluated. This might be acceptable for compendial methods applied to assess general quality attributes in common for several products (e.g. pH, visual inspection, bioburden, endotoxin), but information on product specific analytical methods is essential for the assessment of the applicant's control strategy. Referring to Ph. Eur 2534 (Human coagulation factor VIIa (rDNA) concentrated solution), does not provide the required level of information. Thus, another MO was raised and the applicant was asked to provide enough methodological details to enable the agency to evaluate suitability of respective analytical portfolio to control AS critical quality attributes, within the specific sample matrices.

The applicant presented a summary of performed method validation activities. Acceptance criteria were pre-defined and validation results were listed. Compendial methods were used to assess general quality attributes. Their suitability was demonstrated for the intended use by partial validation studies. No validation protocols and reports were submitted. Thus, the applicant was asked to submit validation protocols and validation reports for the applied compendial and non-compendial methods used for further assessment.

Primary AS batch analysis data, including several consecutive commercial batches and several clinical batches used for clinical trials performed in the EU were presented. All batches complied with the

proposed release specification and no OOS values were found. All quality attributes of presented consecutive commercial batches and of clinical batches were stable and comparable. Nonetheless, it seems that important information was withheld by the applicant. No supplementary information on batches was provided besides release data, manufacturing date and batch numbers. Information on batch scale was found in the AS - Stability Section for some batches only. All these batches did not fulfil the definition of batch volume of the commercial process as claimed by the applicant. Volumes of these batches were considerably lower.

Batch release data of all batches used in the different studies performed trough the dossier has not been included in this section. Thus, information is provided for some batches that were used for the manufacture of the FP batches used in the characterisation exercise and some of them were used for manufacturing process validation. However, data of some batches that were used for the hold time validation studies are not provided in this section. In addition, release data of the AS batches used for the manufacture of the FP batches used in the biosimilarity study have not been included in this section.

The applicant was asked to share batch release data from all manufactured batches, and to provide enough information on batches to enable the authorities to assess if they are representative for the manufacturing process, and to reconsider batch definition. Taken together, the current manufacturing process seems to deliver AS material consistently meeting its specified quality requirements. The applicant is manufacturing rhFVIIa for several years and thus has acquired some manufacturing experience.

AS specification was set based on initial process validation in 2015 and more that 40 AS batches produced for clinical testing in the EU and commercial use outside the EU. When defined by the Ph. Eur. monographs, the applicant implemented exactly these limits for release and stability testing of active substance. This approach was not endorsed, since acceptance criteria should be supported by process performance data, and clinically justified. For all other quality attributes, acceptance criteria were defined based on process capability. Furthermore all specified acceptance ranges must be clinically justified. For the process related impurities, limits were calculated based on the patient exposure levels in the EU clinical trials. This was considered acceptable as long as sufficiently justified by assuming worst case scenarios and suitable safety margins. For the physical and microbiological attributes, the limits have been specified based on common standards concerning injectable biologics. Based on this arguments, the applicant was asked to tighten specification limits.

3.1.2.5. Reference Standard

The applicant has used WHO international FVIIa Reference Standard and EDQM international rhFVIIa for process development, stability programs and AS as well as FP release. In analogy to all other sections of Module 3, only very limited information on reference material was provided. Thus, it is unclear which reference standard was used for which assay. It is also unlikely that only two ampules of international reference standard were sufficient for the whole product development programme including stability studies, and commercial supply for more than 6 years and >40 released batches. It is also unclear which primary Reference Standard currently is applied, while FVIIa Reference Standard being isolated from human plasma definitely is not the right product against which the API shall be compared. The applicant plans to establish an in-house primary Reference Standard and proposed a panel of assays and acceptance criteria. Information on how the in-house Reference Standard was manufactured is missing, and proposed panel of analytical methods is more or less restricted to the ones of AS specification, and respective acceptance criteria are not justified. No information was given regarding the procedure on how the in-house RS was/shall be calibrated. It is also unclear which reference standard is currently applied for which release assays, and how internal reference standards

are manufactured and if such standards have been used yet. The methodological panel used for characterisation is insufficient, and acceptance criteria are not justified. The calibration procedure is not explained. Eventually, stability data show that the primary reference standard is unstable under the proposed storage conditions. Another MO was raised because information on Reference Materials as provided by the applicant is incomplete, and strategy adopted by the applicant is not acceptable.

3.1.2.6. Container Closure System

AS container closure system was sufficiently described. The material in product contact fulfils the requirements of Ph. Eur. 3.1.7. Physicochemical properties and TSE/BSE status are in line with Ph. Eur. 5.2.8. Bags are provided sterile and pyrogen-free by the supplier. The internal QC release procedure and specified acceptance criteria were presented and seem suitable. A down-scaled extractable study simulating a worst-case scenario was performed. A range of solvents, with different physicochemical properties and which cover storage conditions of AryoSeven AS were applied. No non-volatile extractables were found, and no elemental impurities exceeded the permitted daily exposure according to ICH Q3D. Thus, a dedicated leachable study was omitted. Data from a freezing/storage/ thawing study demonstrating AS container closure integrity and resistance to microbial burden under storage conditions should be performed.

3.1.2.7. Stability

Data from long term stability studies performed in a down-scaled model of the primary container closure system are available. The claimed shelf-life is supported, but information required for a proper assessment is missing. Thus, details on the representativeness of used batches, the applicability of the down-scaled model and the analytical methods, the identification of stability indicating parameters, the Reference Standard used to assess potency and the commitment to further stability studies are requested.

3.1.3. Finished Medicinal Product

3.1.3.1. Description of the product and Pharmaceutical Development

A description of the finished product is provided. One dosage form (1.2mg per vial) has been developed. The finished product contains one vial of freeze-dried formulation and one vial of reconstitution solvent (sterile water for injections). The composition of the freeze-dried formulation, i.e., list of all components, their amount per vial and their concentration after reconstitution, the function of the components, and a reference to their quality standards is given.

Furthermore, a brief description of the accompanying reconstitution solvent and the container/closure system used for the freeze-dried formulation and solvent is included.

However, the information on the reconstitution solvent (sterile water for injection) has been split up over the various sections of Module 3 (and not provided as a separate "P" part as requested in the ICH guideline M4Q(R1)). For a finished product supplied with reconstitution solvent(s), the information on the solvent(s) should be provided in a separate part "P", as appropriate. Consequently, the applicant is requested to restructure Module 3 in order to have two separate "P" parts within Module 3: One for the freeze-dried formulation and one for the solvent.

<u>Pharmaceutical development</u>

The finished product is a freeze-dried formulation of a recombinant human coagulation factor VIIa which is co-packaged with a vial containing sterile water for injections as a reconstitution solvent. Regarding the key physicochemical properties of the active substance the applicant refers to CTD section 3.2.S.3.1 Elucidation of Structure and Other Characteristics, but has not identified those properties of the active substance that are clinically relevant for the patient. Consequently, the applicant is asked to briefly discuss those quality attributes that can influence the performance of the finished product and to update CTD section 3.2.P.2.1.1 accordingly.

Regarding the formulation, no novel or unusual excipients have been used. All excipients used in the freeze-dried formulation are of compendial grade. None of the excipients are of human or animal origin.

Interestingly, HemAryo`s recombinant human factor VIIa has been developed as a biosimilar to the reference medicinal product (RMP) NovoSeven from NovoNordisk. A similarity exercise at quality level has been conducted and located in CTD section 3.2.P.2 Pharmaceutical Development. The location of the biosimilarity part in the aforementioned section is not the correct one: The applicant is requested to provide the quality information on biosimilarity in CTD section 3.2.R Regional Information under a separate subheading. Accordingly, the assessment of the biosimilarity part has been placed in chapter "Regional information" within this assessment report.

The applicant indicates that the parameters and corresponding limits have been found suitable according to a CQA based impact assessment supported by extensive commercial scale production experience, but does not provide any evaluation of the criticality of process parameters and in-process testing. No risk-assessment was performed, and the impact on process performance on critical product attributes was not analysed. There is no differentiation between process input and performance parameters, nor between key and critical process parameters. In addition, the given ranges and acceptance criteria have not been justified. Based on the poor level of information provided by the applicant, it cannot be assessed if selected process parameters and acceptance ranges are sufficient to control the production of HemAryo finished product with reproducible quality. Consequently, these issues are included as Major Objection addressing the control strategy of the finished product manufacturing process. The applicant is asked to establish an acceptable control strategy and to completely rewrite this section and to describe this process control strategy according to respective ICH Guidelines Q8(R2) and Q11.

The container closure system used for long-term storage of the finished product and water for injections is a common container closure system used for small volume parenterals. It consists of type I glass vial, a rubber stopper, and an aluminum flip-off cap. The choice of the container closure system has been sufficiently justified. As light stress indicated that the finished product is sensitive towards light exposure, an opaque secondary carton packaging has been used.

The microbial attributes of the unpreserved finished product have been addressed.

No compatibility or sorption studies have been performed between the finished product and any injection and infusion devices. This approach is not fully accepted. The applicant does not give any detailed recommendations regarding the choice of syringes or dosage devices. The applicant is requested to conduct compatibility studies with syringes made of different materials and which are representative for widely used dosage devices on the European market.

3.1.3.2. Manufacture of the product and process controls

The final finished product consists of one vial of freeze-dried formulation (lyophilisate) and one vial of reconstitution solvent (sterile water for injections).

The applicant is requested to update section 3.2.P.3.1 Manufactures as well as the flow-chart (Annex 5.8 in Module 1) with the detailed responsibilities of the different quality control sites. The site responsible to perform each release test should be stated. The description of tests conducted at a specific site must be consistent throughout the dossier (module 3, application form section 2.5.1.2 and flow chart) and the purpose of that testing e.g. in-process control, batch release or stability testing, should be clear. GMP documentation, which confirms the GMP compliance of the finished product manufacturing, testing and batch release sites is included. However, analytical method transfer validation protocols (with pre-defined acceptance criteria) for the biological (potency) assay used at finished product release should be provided before approval for all relevant sites in the MA unless method validation was conducted at the sites in question.

A brief, narrative description of the manufacturing process for the freeze-dried formulation as well as a flow chart, which highlights the main steps in the manufacturing process is included in the dossier. However, a number of shortcomings have been identified in the description of the manufacturing process and its control. The critical steps and points at which relevant process controls (process performance parameters and in-process controls/tests) are performed, should be laid down in the process description. Hold times and durations of specific process steps as mentioned in CTD section 3.2.P.3.4 Control of Critical Steps should be included in the process description. In particular, the presented flow chart is a very high-level overview giving only the main steps without any further details. Apart from the process controls/hold times the steps where materials enter the process need to be highlighted in the flow chart.

The batch formula for the lyophilisate has been given; however, typical batch size ranges including min and max batch sizes with the engaged volume and weight of DS need to be stated.

Concerning the control of critical steps and intermediates a tabulated list of parameters to be controlled, the method used and acceptance criteria are provided.

The applicant indicates that the parameters and corresponding limits have been found suitable according to a CQA based impact assessment supported by extensive commercial scale production experience, but does not provide any evaluation of the criticality of process parameters and in-process testing. In addition, the given ranges and acceptance criteria have not been justified. Based on the poor level of information provided by the applicant, it cannot be assessed if selected process parameters and acceptance ranges are sufficient to control the production of HemAryo finished product with reproducible quality (reference to the Major Objection in 3.2.P.2 Manufacturing Process Development).

An initial finished product manufacturing process validation was performed however no further details from this validation were provided. For the MAA in EU/EEA another process validation was performed which is based on three consecutive finished product batches which were manufactured according to standard operating procedures and within the defined operating ranges. Concerning the process validation it is noticed that process parameters listed in the process parameters and controls for critical steps of the finished product have not been addressed. The applicant was requested to update the validation reports with this information.

Data from qualification and validation studies were not included in the dossier and were required. In addition, there is no information about shipping validation studies, and it has been requested.

Apart from a very brief, superficial summary of the WFI production process, no adequate description of the manufacturing process for the solvent is included in the dossier. Although the production of sterile water for injections is considered simple, the level of information provided for the WFI manufacture is far away from what is expected for a MAA. In addition any information on process validation of the WFI

manufacturing process is completely missing (it remains unclear if a process validation has been conducted at all) a Major Objection is raised on the manufacture of the solvent.

a) As the solvent is used for reconstitution of the lyophilised finished product to achieve a final solution for injection and thus its quality directly affects the patient`s safety, a well-established and controlled manufacturing process for the solvent is essential. For assessment, a well-described manufacturing process needs to be submitted. A flow diagram should be presented giving the steps of the process and showing where materials enter the process. The critical steps and points at which process controls, intermediate tests or final product controls are conducted should be identified. A narrative description of the manufacturing process that represents the sequence of steps undertaken and the scale of production should also be provided. Equipment should, at least, be identified by type and working capacity, where relevant. Steps in the process should have the appropriate process parameters identified.

b) Description, documentation, and results of the validation and/or evaluation studies should be provided for critical steps or critical assays used in the manufacturing process. The results from the validation work should confirm that the WFI production process is capable to produce consistently solvent meeting its predefined quality requirements.

Furthermore, no information on the batch size of the solvent is given. Thus, the nominal batch size for the solvent in the vial should be provided.

3.1.3.3. Product specification, analytical procedures, batch analysis and excipients

Control of Excipients:

Widely used excipients, which comply with the respective Ph. Eur. monographs, are used for formulation of the final finished product. Section 3.2.P.4.4 Justifications of Specifications contains only very general comments, but does not discuss the rationale for the selected specifications and acceptance criteria for control of one of the excipients.

Control of Drug Product:

For control of the formulation as well as for the solvent specifications have been presented. The release specifications for the freeze-dried formulation include tests for identity and potency, impurities, microbial contamination, protein content and general quality attributes. A subset of these tests is applied for stability testing. Is principally agreed that relevant quality attributes are proposed to be controlled at a routine basis; however, several concerns are noted which leave some uncertainties open.

No tests for content of the excipients is included. This not acceptable. Validated analytical methods for routine control of excipients content should be included.

Testing of certain finished product quality attributes were not included into the stability testing. The applicant`s reasoning that these quality attributes are not stability indicating for a lyophilised formulation cannot be completely followed. The applicant is requested to demonstrate that these attributes are indeed not relevant for long-term stability.

Concerning the information on the analytical methods to be included in Module 3 it is usually agreed that a brief description including the method principle suffices for a MAA and that no excessive details, SOPs or equivalent is expected. However, for certain analytical methods the information provided is extremely limited and essential core information (protocols, used reagents, buffers, media, columns and their provenience, and how raw data are processed and evaluated) is missing. Thus, the applicant is asked to provide enough methodological information to enable the Agency to evaluate the suitability

of the respective analytical portfolio to control finished product critical quality attributes, within the specific sample matrices. Reference to the respective Major Objection in the active substance section is made (MO).

It should be also noted that certain quality attributes, which have been monitored during development are not included in the finally proposed specifications. The provided justifications for omission of release and stability testing for these quality attributes can be accepted.

Regarding the method validation only brief, tabulated summaries of the conducted validation work have been presented, but no details are available. Consequently, the applicant is requested to submit method validation reports in order to enable an in-depth assessment and conclusion if the applied methods are indeed suitable for their use. In addition, the applicability of compendial methods to measure safety related parameters needs to be confirmed in the respective sample matrix. Thus, the applicant is asked to confirm matrix validation for compendial methods used to assess FP safety related parameters.

Regarding the batch analyses the applicant has provided the release results from only a limited subset of the available finished product batch data. It is noted that the analysis results from the process validation batches as well as from the batches used in EU clinical trials have been provided. However, the applicant is requested to provide all batch analysis results which are considered relevant for the finished product development. For a considerable number of batches the release results have not been included. Consequently, the applicant is requested to update the batch analyses section with the results from all finished product batches, which are considered relevant for the finished product development and the setting of release specifications.

Characterisation of impurities, which may occur at finished product level, has been briefly addressed. However, a summary of the risk assessment for elemental impurities in accordance with ICH Q3D should be included in the dossier. A risk analysis to identify potential FP process-related impurities coming from materials in contact with the medicinal product (filters, plastic connections, intermediate containers, CCS) should be presented. The information should be included Section P.5.5 Characterisation of impurities.

With respect to the finished product release control, justifications for the chosen specification acceptance criteria have been presented. Release acceptance criteria for non-stability indicating quality attributes have been based on process capability, on historical manufacturing experience with the finished product and/or compendial requirements for injectables. For stability indicating parameters the release and shelf-life limits have been established by taking into account the active substance acceptance criteria, finished product process contribution and the proposed finished product shelf life. This rational is not fully understood/agreed upon and further justification is requested. Nevertheless, certain acceptance criteria seem to be rather wide and recalculation/tightening is requested.

Finally, it is obvious that during development, the acceptance ranges for a number of release and stability tests have been changed. For the release criteria a tabulated summary of the changes implemented during development including the justification for the changes is requested. For a number of quality attributes it seems that the release acceptance criteria have been widened during development. This widening of acceptance criteria during later phases of development is usually not accepted as it is expected that the manufacturing process as well as the process control strategy will be continuously improved leading to a high-quality product. Taking these considerations into account it is not understood why at the stage of a MAA much wider release acceptance criteria are in place when compared with the acceptance criteria valid at earlier development steps. The applicant is requested to elaborate on this issue.

Regarding the control of the solvent, the specification has been established according to Ph. Eur. requirements for sterile water for injections. Furthermore, the test for extractable volume is performed to ensure that the labelled nominal volume can be withdrawn for reconstitution followed by appropriate dose preparation. Release results of three representative Water for Injections batches are presented. However, it is not clear if these batches represent the process validation batches or batches used in the clinical trial. The applicant is requested to update this section with batch release data from clinical and process validation batches.

3.1.3.4. Container closure and stability of the product

Container Closure System

The container closure system chosen for long-term storage of the freeze-dried formulation and WFI comprises a type I glass vial, a rubber stopper, and an aluminium flip-off cap. A description of the container closure components has been provided. The specifications for each component are given and also include critical dimensions; the glass vials as well as the rubber stoppers comply with the respective Eur. Ph. monographs. In addition, technical drawings of each single container closure component are given. Finally, a brief description of the secondary packaging (which protects the finished product vials against the light and the damage during transport, handling and storage) is also given. Nevertheless, the suppliers of container closure systems (e.g. rubber stoppers, glass vials and caps) and representative supplier CoA`s should be provided.

In addition to the description of the container closure components extractables and leachables have been briefly discussed in this section. The applicant has conducted an extractable study. Finally, an elemental impurity screening in samples will be conducted. The results will be evaluated against permitted concentration of elemental impurities as listed in Table A.2.1 in ICH Q3D. No results from the leachable studies are currently available. Furthermore it is expected that a risk assessment for all materials in contact with product during all steps of the manufacturing process will be performed: For high-risk materials, extractable/leachable studies should be initiated. Due to these issues raised above an assessment and conclusion on acceptability of the extractables and leachables section is currently not possible. To address this uncertainty, the applicant is requested to provide

a) at least an interim report of the ongoing leachable studies and elemental impurity screening studies on the rubber stopper which summarises the results from these ongoing studies as far as they are available and

b) conduct a risk assessment for all materials in contact with product during all steps of the manufacturing process and

c) initiate extractable/leachable studies for high-risk materials and provide the results thereof.

Stability of the Finished Product

The claimed shelf life for the finished product is based on available long-term stability data from several lyophilised product batches, which have been manufactured at the large-scale manufacturing facility for commercial product according to the validated FP manufacturing process. Several lyophilised product batches were manufactured consecutively in one campaign and several additional lyophilised product batches, stored during the stability study in upside-down position, were manufactured afterwards at approximately one-year interval. Studies are completed for some lyophilised product batches and still ongoing for the remaining batches. In addition, accelerated studies were performed

on the lyophilised product batches manufactured consecutively in one campaign. The container closure system used for stability studies of the lyophilised product and WFI is identical to that intended for the market.

Although the amount of stability data/number of lyophilised product batches put on stability may be sufficient for the claimed shelf-life, a number of issues have been identified which currently preclude the proposed shelf-life.

It remains unclear which shelf-life specifications are in place for the long-term stability testing due to discrepant information given in the dossier. In particular, in CTD section 3.2.P.5.1 shelf-life acceptance limits for a number of quality attributes are different (in most cases much wider) than the shelf-life specifications mentioned in CTD section 3.2.P.8.1. This widening of shelf-life acceptance criteria after conduct of the primary stability studies is not acceptable.

Furthermore, the shelf-life specifications have been changed during the primary stability studies. The applicant is requested to clearly state the shelf-life specifications and to correct the respective sections in the dossier accordingly. In addition, the implemented changes/shifts of the shelf-life specifications should be justified and their impact on the validity of the primary stability studies needs to be discussed.

Photostability testing was performed. The photostability design presented is incomplete because, as described in ICH guideline Q1B, samples must be exposed to both cool white fluorescent and near ultraviolet lamps, which should be appropriately described.

The applicant has also provided the stability information for its water for injections used as solvent for preparation of the reconstituted solution. This self life claim is based on stability data from several solvent batches put on long-term stability. The available stability data can be considered sufficient for the proposed shelf-life claim for the solvent.

3.1.3.5. Biosimilarity

HemAryo (approved as AryoSeven in Iran) 1.2 mg (60 KIU) – powder and solvent for solution for injection has been developed as a biosimilar to the reference medicinal product (RMP) NovoSeven from NovoNordisk which was introduced to the European market in 1996. NovoSeven was initially provided as a cold storage freeze-dried formulation. Since 2008, a new NovoSeven formulation was introduced to the European market which differs in formulation and in active substance amount per vial (from 1.2 mg to 1.0 mg). In addition, the solvent for reconstitution was changed (change from WFI to a histidine buffer). This new formulation offered a storage condition at room temperature.

Due to patent restrictions on the new formulation, the HemAyro finished product has been developed with reference to the old NovoSeven formulation, which is not on the market anymore. Due to difficulties with availability of the old 1.2 mg presentation of RMP the applicant used the 1 mg presentation of RMP with the new formulation which is currently on the market for the conduct of the biosimilarity exercise. This issue was extensively discussed in a previous EMA scientific advice procedure. In addition, reference to EMA Guideline CHMP/437/04 Rev1 was made which allows deviations of the biosimilar from the reference product as regards strength, pharmaceutical form, formulation, excipients or presentation if justified and if it does not have a relevant clinical effect or compromise safety. Taking these arguments into account, no principal concern was raised on this aspect. Nevertheless, the applicant is requested to discuss how potential dosing errors due to the different strengths will be addressed and to provide an outlook if and how the different strengths between biosimilar and RMP will be aligned.

The biosimilarity evaluation was performed as a head-to-head comparison between NovoSeven (in the current marketed formulation) and HemAryo. Several batches of each NovoSeven and HemAryo were used for these studies.

Following quality attributes were compared: Sequence and post-translational modifications; sialic acids as well as monosaccharides/ glycosylation analysis; N-glycan profiling and linkage analysis; N-glycosylation site determination; secondary structures; secondary and tertiary structures; thermal characteristics; disulfide linkage and unlinked cysteines; extinctions coefficient; molecular weight or size; protein aggregation and degradation; subvisible particle analysis; non-activated rhFVIIa (Single chain); oxidised and degraded forms; Gla-domainless forms; biological activity.

In addition, a forced degradation study was designed and performed to simulate and demonstrate the possible formation of product-related impurities in HemAryo. To evaluate the impurities formed in HemAryo during forced degradation, NovoSeven was also included in these studies.

However, the presented biosimilarity exercise is far away from what is usually expected for a large, recombinant protein with multifaceted post-translational modifications and a complex mode-of action. Several major deficiencies have been identified which currently preclude a biosimilarity claim of HemAryo to NovoSeven (Major Objection).

- Inclusion of only five RMP batches into the whole biosimilarity evaluation is far too limited to give an impression on the variability of the RMP on the market. In addition, it remains unclear if these five RMP batches have been sourced from the EU market. As outlined in the EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) multiple different batches of the reference medicinal product should be used to provide robust comparability data in order to generate a representative quality profile. Consequently, the applicant is requested to analyse additional batches of the RMP in order to establish the quality target product profile (QTPP) of the biosimilar. The combined use of a non-EU authorised and EU authorised reference product is acceptable for the development of a QTPP. However, for demonstration of biosimilar comparability at the quality level side-by-side analysis of the biosimilar product (from commercial scale and site) with EEA authorised reference product must be conducted.
- In addition, the number of HemAryo batches included in the biosimilarity exercise is quite limited considering the long manufacturing history of HemAryo (since the conducted process validation for a MAA in the EU more than 34 batches have been manufactured). The selection of these five HemAyro batches has not been justified and its remains unclear if a predefined sampling strategy has been in place. It seems that the currently selected HemAryo batches represent batches used in the clinical studies, but neither the full set of clinical batches nor process validation batches have been included for biosimilarity evaluation. The applicant is requested to include at least the missing clinical as well as the process validation HemAyro batches into the biosimilarity evaluation.
- No criticality assessment of the quality attributes of the rhFIIa molecule has been presented. Without this essential information, it remains difficult to conclude if certain differences have to be considered relevant or not. In fact, certain differences have been observed, but not further discussed. It remains unclear if these differences have any impact on the efficacy and safety profile of the product. Consequently, the applicant is requested to carefully analyse the potential impact of each quality attribute on safety/immunogenicity and efficacy via risk assessment tools in line with ICH Q8 Pharmaceutical Development (2009) and ICH Q9 Quality Risk Management (2005). Based on the outcome of this risk assessment a criticality should be assigned for each studied quality attribute. Subsequently, a discussion and justification of detected quality differences taking the criticality of the affected quality attribute into account needs to be submitted.

- It remains completely unclear if similarity ranges for quantitative quality attributes have been established and on which criteria similarity has been concluded. The applicant is requested to describe how similarity ranges have been set and to update the biosimilarity part with the ranges for each quality attribute. The applicant is referred to the updated and final version of the Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development (EMA/CHMP/138502/ 2017). The use of statistical approaches to conclude on comparability is generally endorsed and the pre-specification of any rules to decide upon comparability for a specific attribute (including a discussion which difference would be relevant in terms of possible impact on clinical outcome) will enhance the credibility of the findings.
- For comparative characterisation of the biological functions only two assays were used. Considering the complex mode of action for rhFVIIa and its multifaceted interaction with different molecules during the blood coagulation, the set of two proposed assays is too limited to gain a full picture of the biological activities of rhFVIIa. The applicant is requested to amend the panel of assays for biological characterisation with additional assays in order to evaluate and compare the full functionality of rhFVIIa within the coagulation cascade.
- The applicant indicated that all applied analytical methods were validated and/or qualified for the used purpose, but did not provide any qualification/validation reports demonstrating that these methods are indeed suitable for their use. Submission of these reports or at least of assessable summaries is requested.

Apart from this major objection on the principal conduct of the biosimilarity exercise a high number of other concerns has been raised.

Although classified as Other Concerns, these numerous issues add additional uncertainty on the performed biosimilarity exercise. In conclusion, the points raised in the Major Objection together with the relatively high number of additional other concerns leave some doubts on the applicant's principal understanding of a biosimilarity approach. From the Assessor`s understanding the raised issues in the major objection cannot be easily solved. Thus, the obviously best way to address the serious issues would be to repeat the biosimilarity exercise taking the discussion of the points in the Major Objection into account.

3.1.3.6. Post approval change management protocol(s)

Not applicable.

3.1.3.7. Adventitious agents

The applicant has briefly discussed the section on viral adventitious agents. Four primary sources have been identified which may act as sources for virus contamination.

Regarding the virus validation studies the applicant has identified several steps in the purification process to have viral clearance capabilities. These steps were investigated for their potential for virus removal or inactivation. It is agreed that the model viruses selected represent potential contaminants of the product and cover a wide range of physico-chemical properties. The presented reduction factors indicate that the established manufacturing process provides an adequate viral safety assurance with several logs of safety margin. However, the main issue here is that only a very brief, superficial summary of the conducted virus validation studies has been included. Essential core information on the design, conduct and outcome of the virus validation studies has not been provided; thus making an assessment of these validation studies impossible. The issues raised in this section are included in a Major Objection on viral safety. Consequently, the applicant is requested to restructure the section on Adventitious viruses and provide additional information such as:

- An adequate and clear description of the downstream models used for the virus validation studies needs to be submitted. Important process parameters need to be compared and convincingly reproduced in the down scale process.
- Verification that the downstream models used for virus clearance are indeed representative of the commercial process is requested.
- Section 3.2.A.2 should include comprehensive summaries of the performed virus validation studies with sufficient information to conclude on the acceptability of the performed virus validation experiments. The individual virus validation reports with the appropriate details should be also included as Annexes to the summary.

3.1.3.8. GMO

Not applicable.

3.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

HemAryo (rFVIIa) is a 50 kDa glycoprotein produced by a genetically transformed Baby Hamster Kidney (BHK) cell line and purified by consecutive purification steps. Recombinant FVII is expressed as a single chain protein, which during purification gets activated by proteolytic cleavage into the activated form denoted rFVIIa. FP composition is identical to the AS. Thus, no further manufacturing step besides thawing, filtration, pooling, filling and lyophilisation is required for the production of the FP.

Module 3 was of poor quality and the applicant only gave high level overviews and summaries, whose content would even not be acceptable for Module 2. Besides essential activities and studies which were not performed at all, specific information in many cases was missing. If at all, only selected and partial results were presented without legends, explanation or conclusions, and they were not discussed. The assessment of the suitability of the applicant's CMC activities was mostly impossible, resulting in several MOs.

The production process was not sufficiently described, leaving many manufacturing steps unclear. The active substance was not sufficiently characterised. The selected data provided by the applicant show that many product- and process-related impurities are present in the AS, but they were not addressed, nor further identified or quantified. The applicant did not elaborate the target product quality profile and critical quality attributes impacting on product pharmacology, activity and safety. Proposed in-process controls consisted of a list of some selected process parameters, without any link to CQAs. No quality risk assessment was performed, and many process parameters including process performance and in-process homogeneity were not studied at all. Acceptance criteria for process controls were broad and unjustified. Thus, process validation activities as performed by the applicant did not demonstrate that the manufacturing process is able to reproducibly produce rhFVIIa of a defined quality. Most acceptance criteria for raw-materials and materials of non-compendial origin and their proveniences were not provided. Process development activities for several manufacturing steps were sufficiently described by the applicant. AS specification only insufficiently addresses CQAs, since respective IPCs are not in place, and since AS specification is based on respective Ph. Eur. rhVIIa monography only, without addressing any product specific micro-heterogeneity and process related impurities. No details on analytical procedures and their validation were given, and thus assessment of their suitability was not possible. Specification limits were set based on rhFVIIa monography and process performance, but need to be clinically justified and adjusted according to batch analysis data of respective clinical lots used in EU-trials. Information on Reference Standard and its provenience was

incomplete. Furthermore, proposed analytical qualification is incomplete and the reference standard seems to be unstable. AS container closure system seems qualified, but its suitability should also be addressed under long term storage conditions. No nitrosamine risk assessment was performed.

The same comments highlighting the general issues with the active substance part of Module 3 are also valid for the finished product part. A poor dossier which is partly far away from what is expected for a MAA in Europa. For many sections only high-level overviews and summaries are presented whereas core information and essential details required for an assessment are missing. Furthermore, serious discrepancies and non-alignment of the information presented in the different sections are noticed. Finally, only selected and partial results were presented without legends, explanation or conclusions, not discussed. Consequently, these issues resulted in several MOs addressing the control strategy of the finished product manufacturing, the manufacture of the solvent, the viral safety and finally the performed biosimilarity exercise. In addition, a number of other concerns related to the various sections of the finished product part are raised.

3.2. Non-clinical aspects

3.2.1. Introduction

In support of the non-clinical development of HemAryo no study reports but only bibliographical data have been submitted.

3.2.2. Pharmacology

3.2.2.1. Primary pharmacodynamic studies

In order to evaluate the similarity of HemAryo and NovoSeven, literature references on **comparative *in vitro* studies** were provided. Briefly, the assays included a **functional FVII assay** (Sadeghi 2014) and the measurement of **procoagulant activity** (Sadeghi 2014), both using a clot-based method, and the **thrombin activation** profile by SDS-PAGE and Immunoblotting (Sadeghi 2017). No *in vivo* pharmacodynamics studies were conducted.

In the **functional FVII assay** FVII-deficient human plasma was supplemented with rFVII to yield a concentration of 100ng/ml. After addition of a recombinant human TF to induce coagulation the time to clot formation was recorded. Normal human plasma was used to generate a standard curve and assuming the 1:10 dilution as 100% concentration, clotting time versus % concentration of FVIIa was used to extrapolate percent concentration of rFVIIa in the samples. The mean concentration showed no statistically significant difference (p= 0.3094) in % concentration between NovoSeven® and eptacog alfa (AryoSeven), although the biosimilar candidate eptagog alfa showed a slightly higher value. The FVII/VIIa levels were about 60%-75% of the normal plasma value for both groups.

Prothrombin time (PT) and **activated partial thromboplastin time (aPTT)** were determined in human whole blood and retrieved plasma supplemented with 1, 0.5, and 0.25 µg/mL of rFVIIa. PT was investigated as well in FVII-deficient plasma supplemented with both types of FVIIa at levels of 1 to 0.001 mg/mL using two different thromboplastin reagents (RecombiPlasTin and Innovin), latter not considered necessary but acknowledged. Actin FSL reagent was utilised for aPTT measurement, whereas it is not clear which type of tissue factor was used for PT measurement in the first set of experiments in human whole blood and retrieved plasma. The results of these studies showed that eptagog alfa (Aryoseven) and NovoSeven® had comparable pharmacodynamic effects on the clotting profile of human whole blood and retrieved plasma.

The ability of the **generation of thrombin** was comparatively investigated with Aryoseven and NovoSeven (EEA-authorised batches) in *in vitro* experimental studies utilising two different Protein complex concentrates (PCCs), activated by tissue factor recombiplastin (RCP). These comparative studies included sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and immunoblotting studies utilising a rabbit anti-human recombinant thrombin antibody. In the first set of experiments, activated PCCs were supplemented with 2 different concentrations of rFVIIa to give a final concentration of 0.25 and 0.5 µg/mL and incubated for 30 minutes. To reveal an effect of incubation time on the generation of thrombin with a fixed final concentration of rFVIIa (0.1 µg/mL), SDS-PAGE and immunoblotting was carried out as a second set of experiment using incubation time points of 5 and 15 minutes. It is to note that different batches of Novoseven and Aryoseven were utilised in the two sets of experiments (i.e. set 1: 2 different concentrations of rFVIIa at 30 minutes; set 2: one concentration of rFVIIa at 5- and 15 -minutes incubation time). In the first set of experiments gel electrophoresis revealed that rFVIIa improved the generation of thrombin in activated Profilnine, the 3-factor PCC. Neither concentration- nor product-dependent differences were observed. Similar results were obtained with Beriplex, the 4-factor PCC. But in contrast to the experiment with Profilnine, addition of rFVIIa to Beriplex showed no enhancement of generation of thrombin from prethrombin in comparison to activated Beriplex alone. In the second set of experiments shorter incubation time prevented complete conversion of prethrombin to thrombin in the presence of rFVIIa upon supplementation in activated Profilnine. Intensity of prethrombin bands was comparable between both rFVIIa productsand higher in 5-minutes incubation time than in 15-minutes incubation time. The same study was conducted with Beriplex as PCC. Neither differences in protein profile with different incubation times was detected, nor any differences were observed between NovoSeven and Aryoseven. But in contrast to the experiment with Profilnine, addition of rFVIIa to Beriplex showed no difference to the activated PCC alone.

3.2.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were conducted.

3.2.2.3. Safety pharmacology programme

No non-clinical safety pharmacology studies were conducted.

3.2.2.4. Pharmacodynamic drug interactions

The **pharmacodynamics drug interaction** study from Lauritzen (2019) investigated the concomitant **effect of rFVIIa NovoSeven and Concizumab**, a potential monoclonal antibody (mAb) against tissue factor pathway inhibitor (TFPI), which was in clinical development as a subcutaneous prophylactic therapy for haemophilia A/B with and without inhibitors. It is to mention that the clinical studies with Concizumab were early terminated due to possible safety issues. Furthermore, in this non-clinical study, no data were produced for Ayroseven to be comparatively assessed with NovoSeven. **Human blood made haemophilic** with a FVIII antibody was spiked with increasing concentrations of concizumab, rFVIIa, or concizumab and rFVIIa in combination, and this was followed by **thrombin generation test or thromboelastography**. In human blood, concizumab + rFVIIa had more pronounced procoagulant effects under haemophilic conditions than the individual responses. **Blood loss in haemophilic rabbits** was measured when concizumab, rFVIIa or concizumab + rFVIIa was administered either before or during cuticle bleeding. In contrast to the human blood study, concizumab + rFVIIa had no additional effects on blood loss in haemophilic rabbits as compared with rFVIIa or concizumab alone. **Cynomolgus monkeys** were exposed to high steady-state concizumab concentrations and given three doses of rFVIIa, and then subjected to full necropsy and histopathological examination. The macroscopic and microscopic pathological examinations revealed no

thrombi or other signs of excessive coagulation activation. Both rFVIIa and concizumab caused increases in thrombin– antithrombin and D-dimer concentrations; this effect tended to be additive with concomitant administration.

Pharmacodynamic interaction has been also reported **combining rFXIII and rFVIIa** (NovoSeven). A potential synergistic effect of combined treatment with rFXIII and rFVIIa in an advanced cardiovascular model in **cynomolgus monkey** resulted in exaggerated pharmacology (thrombosis and death) at a lower dose level than when administering the individual compounds. Based on this non-clinical study it is not recommended to combine rFVIIa and rFXIII. There are no clinical data available on interaction between rFVIIa and rFXIII (SmPC NovoSeven).

3.2.3. Pharmacokinetics

Non-clinical studies to support the pharmacokinetics to support the biosimilar application of eptacog alfa have not been performed.

3.2.4. Toxicology

Non-clinical studies to support the toxicology to support the biosimilar application of eptacog alfa have not been performed.

3.2.4.1. Single dose toxicity

N/A

3.2.4.2. Repeat dose toxicity

N/A

3.2.4.3. Genotoxicity

N/A

3.2.4.4. Carcinogenicity

N/A

3.2.4.5. Reproductive and developmental toxicity

N/A

3.2.4.6. Toxicokinetic data

N/A

3.2.4.7. Tolerance

N/A

3.2.4.8. Other toxicity studies

N/A

3.2.5. Ecotoxicity/environmental risk assessment

The current application for HemAryo, Eptacog Alfa (activated), is submitted as a Biosimilar application in accordance with Article 10(4) of Directive 2001/83/EC, as amended. HemAryo is a powder and solvent for solution for injection. The European reference product is Novoseven that was authorised the 23rd of February 1996.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Eptacog alfa is already used in existing marketed products and no significant increase in environmental exposure is anticipated.

Therefore, HemAryo is not expected to pose a risk to the environment.

3.2.6. Discussion on non-clinical aspects

As in line with the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1), no *in vivo* pharmacodynamics studies were performed under the assumption that the biosimilar comparability exercise for the physicochemical and biological characteristics and the non-clinical *in vitro* studies are considered satisfactory.

Since severe deficiencies have been identified in the ***in vitro*** **pharmacodynamic** part of the non-clinical dossier, an appropriate comparative assessment of HemAryo and the reference product is not possible. The applicant did not follow the recommendations laid down in EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1) to allow a thorough biosimilar assessment. In particular, the submitted study results on *in vitro* PD are not considered to be sufficiently sensitive and discriminatory to detect potential differences between the biosimilar candidate and the comparator. Furthermore the deficiencies, detected in the bioanalytics part as well as in the clinical PD part of the dossier, put even more emphasis on the non-clinical *in vitro* PD assessment.

The applicant is asked to address the following major deficiencies:

a. Insufficient *in vitro* data demonstrating a concentration-activity relationship of HemAryo and the comparator have been provided. Thus, a dose-response was only shown for one coagulation parameter, i.e. prothrombin time, in FVIIa-deficient plasma. However, these results were presented solely pooled by product and as a graph; no information on the number of different samples, replicates, etc. was provided. The decrease in PT was approximately of the same amplitude at the three dose levels tested in whole blood and in retrieved plasma. Furthermore, it is to note that the difference in clotting time between the controls and samples was very small or even absent. It appears that clotting time (with either PT or aPPT) might not be sensitive enough to highlight differences between the two rhFVIIa preparations. Thus, the appropriateness of the experimental setting for the identification of small differences between the two products is considered questionable. Also, no dose-response was established in the functional FVII assay in FVII-deficient plasma that involved only one concentration of each FVIIa-product, HemAryo and Novoseven. For comparative purposes a wider concentration range would have been appropriate. Other, more sensitive functional assays could be carried out instead, so as to measure thrombin generation time parameters, binding to TF, FIX/FX activation, or even kinetic inhibition by antithrombin.

b. For the clot-based non-clinical biosimilar exercise exclusively US batches of NovoSeven were used as references. According to the guideline on similar biological medicinal products (CHMP/437/04 Rev 1, 23 October 2014), for demonstration of biosimilar comparability -, side-by-side analysis of the biosimilar product (from commercial scale and site) with EEA authorised reference product must be conducted.

c. Thrombin generation after a fixed incubation time was comparatively assessed in two different prothrombin complex concentrates by visual evaluation of SDS-PAGE and Western Blots, which are considered qualitative in nature and not sensitive enough for a biosimilar assessment. These assays are a qualitative characterisation of signal transduction and should not be used for establishing a dose-response. Rather the effect of various FVII-concentrations should be comparatively assessed over time with parameters such as lag time, time to peak, thrombin peak and endogenous thrombin potential being evaluated.

d. *In vitro* PD assays were predominantly performed with normal human blood or plasma and PCCs, respectively, whereas FVII-deficient plasma was only scarcely included in the comparative assays. It is acknowledged that according to EMA Guideline on similar biological medicinal products (CHMP/437/04 Rev 1, 23 October 2014), "if biosimilarity has been demonstrated in one indication, extrapolation to other indications of the reference product could be acceptable with appropriate scientific justification." However, the limited scope of experiments conducted together with a missing discussion on the relevance of the study results for Haemophilia A and B patients with and without inhibitors represents a considerable uncertainty with regard to the requested indications.

It is acknowledged and accepted that no **secondary pharmacodynamics** studies were conducted with HemAryo.

No non-clinical **safety pharmacology** studies were conducted which is accepted, as they are not required for the non-clinical testing of biosimilars according to the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1).

In line with the application as a biosimilar no studies on **pharmacokinetics** were conducted for HemAryo. Pharmacokinetic parameters have been evaluated in the scope of clinical trials.

The applicant did not perform studies on **toxicology** which is acceptable when following the step-wise approach laid down in EMA guideline EMEA/CHMP/BMWP/42832/2005 Rev1.

It is agreed that no studies on **Environmental Risk Assessment** are necessary for HemAryo due to its nature as a protein.

3.2.7. Conclusion on non-clinical aspects

The *in vitro* functional evidence for similarity of HemAryo to the comparator Novoseven submitted in the non-clinical part of the dossier exhibits major deficiencies and is therefore considered insufficient for MAA. A non-clinical Major Objection on the *in vitro* pharmacology has been raised.

3.3. Clinical aspects

- **Tabular overview of clinical studies**

Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test product: Dosage regimen: Route of	Numbe r. of Subjec ts	Healthy Subjects or Diagnosis,	Duration of treatmen t	Study Status; Type of Report
PK/PD Safety , Efficacy	UGA 2014-01	PK, PD bioequivalence with NovoSeven Efficacy, Safety including immunogenicity	Double blind, randomised, cross-over. Open, 12 months	PK/PD, cross over 90µg/kg or 270 µg/kg iv Treatment of bleeding 90µg/kg, one or more iv	48 (PK/P D) 42 open follo w- up	Haemophi lia A/B with inhibitors	Single dose cross-over; 1 to 3 or more doses	Ongoing, Full report for PK/PD phase
PK Safety , Efficacy	UGA 2014-02	PK bioequivalence with NovoSeven Efficacy, Safety including immunogenicity	Double blind, randomised, cross-over. Open, 12 months	PK cross over 30µg/kg iv Treatment of bleeding 30µg/kg, one or more iv injections	24	Factor VII Deficienc y (FVII<1%)	Single dose, cross-over; 1 to 3 or more doses	Completed, full report
Efficac y, Safety	IRCT2012021 06302N2	Compare clinical efficacy Compare	Double blind, randomised,	90µg/kg; 120µg/kg after 2h; 120µg/kg after 2h,	66	Haemophi lia A/B with inhibitor	3 doses per bleedin g	Completed, full report
Efficacy Safety	IRCT201202 106302N1	Comparabe plasma levels of FVII:C 20 min after dosing Compare frequency and severity of bleeding	Double blind, randomised,	30µg/kg iv a week for 4 weeks	66	Factor VII Deficiency	4 weeks	Completed, full report
Safety	ARY2016-01	Immunogenic ity Adverse events	Observationa l, prospective,	Various, depending on disease	218	Various, treated with	Various, not defined	Completed, full report

3.3.1. Clinical pharmacology

3.3.1.1. Pharmacokinetics

Introduction

Pharmacokinetic comparability data has been generated in two clinical trials (**Study UGA 2014-01** and **UGA 2014-02**) in patients with haemophilia A/B with inhibitors and patients with congenital factor VII deficiency (<1%), respectively. Both studies had the scope of showing bioequivalence with EU-Novoseven. Three doses have been tested: 30 µg/kg (UGA 2014-02), 90 µg/kg and 270 µg/kg (UGA 2014-01).

Analytical Methods

PK and PD Assays

Bleeding episodes in haemophilia A and B inhibitor and Congenital factor VII (FVII) deficiency patients are frequently treated with recombinant activated factor VII. Pharmacokinetic assessments needs to be performed with an assay able to discriminate between FVII/FVIIa and rFVIIa. Thus the commercially available Staclot® VIIa–recombinant tissue factor assay was used. The most important validation parameters were sufficiently addressed in a partial validation, but demonstration of accuracy, dilution

linearity and selectivity need to be repeated by using the right matrix. PD effects of rFVIIa on coagulation and fibrinolysis was studied by testing parameters as F1.2 prothrombin fragments (F1.2 or F1+2) and D-dimer. These markers have been previously reported to be slightly elevated following rFVIIa administration but without any adverse clinical consequence. Also the Thrombin Generation Assay (TGA) is a very useful tool that provides a more comprehensive clot assessment than the prothrombin time (PT) and activated partial thromboplastin time (aPTT), which measures one-dimensional time to clot formation. Commercial assay-kits for the D-Dimer, TGA and F1+2 determination (PD parameters) in human plasma samples were partially validated for their intra-assay precision and accuracy by the European Research Biology Center, Italy. The applicant was asked to disclose the matrix and the acceptance criteria of the calibration curve in both assays. The Abbexa human prothrombin fragment F1.2 ELISA kit is a sandwich ELISA Kit against Prothrombin Fragment 1+2, for research purpose. It was partially validated based on pre-defined acceptance criteria for its range, precision and accuracy, but should be fully validated according to EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2 and ICH Q2 (R1) guideline. No incurred samples reanalysis (ISR) appears to have been performed for the FVIIa clotting activity assay in human. Given the pivotal nature of the bioequivalence PK-PD studies and as outlined by the guideline EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2, ISR should be done in such case. Hence, the applicant is requested to provide these data.

UGA 2014-01

Methods

The study was a Phase III, multicentre, randomised, double-blind, two-dose, two-ways, cross-over bioequivalence clinical trial to compare the PK and PD of HemAryo and NovoSeven after a single iv. injection in adult and paediatric patients (>12 years) with haemophilia A or B with inhibitors titer >5 Bethesda Units [BU] and with > 2 episodes of bleeding requiring treatment with FVII infusions within the previous 12 months at trial start, not in bleeding status.

Figure 1

(A) AryoSeven 90 µg/kg; (B) NovoSeven 90 µg/kg, (C) AryoSeven 270 µg/kg, (D) NovoSeven 270 µg/kg,

UGA 2014-01 consists of a first phase for the evaluation of PK and PD in a cross-over design using EU-NovoSeven as reference (Phase A) and a second phase for the evaluation of the clinical response and immunogenicity in a single arm design without comparator (Phase B).

The primary objective of this study was to demonstrate biosimilarity between the biosimilar candidate HemAryo and NovoSeven based on PK and PD in patients with congenital haemophilia A/B with inhibitors >5 Bethesda Units [BU] .

The secondary objectives were to evaluate additional PK parameters, to assess clinical response in controlling acute bleeding, to evaluate safety (AEs) and immunogenicity.

A total of 48 patients were enrolled to be randomly assigned to receive two injections (either two times 90 µg/kg or two times 270 µg/kg) separated by a washout period of 3 days.

At the end of the PK/PD phase, all patients entered an open phase of 12 months. For every bleeding episode that occurred during the 12 months, patients received AryoSeven 90 µg/kg for one or more doses until resolution of bleeding (the dose and duration of treatment were based on the Investigator's decision), and were assessed for clinical efficacy, monitoring of inhibiting antibody formation, and collection of safety data.

Study Participants

Inclusion Criteria

- Male subjects >12 years of age
- Confirmed diagnosis of congenital haemophilia A or B with inhibitors to FVIII or FIX titer >5 Bethesda Units [BU], with > 2 episodes of bleeding/year requiring treatment with FVII infusions, non in bleeding status
- Voluntary written informed consent to the protocol to be eligible for the study
- For minor patients, the parent/legal guardian gave consent and, when possible, patient assent was obtained. For compromised patients, their designated proxy must have provided informed consent.

Exclusion Criteria

- Any other type of congenital or acquired coagulopathy such as liver disease (hepatitis), vitamin k deficiency, uraemia, malignancy
- Antibodies against Factor VII
- Ongoing bleeding prophylaxis with NovoSeven regimens or planned to occur during the trial
- Patients who have received routine (prophylactic) treatment with rFVIIa in the period between screening visit (visit 1) and visit 2 of this study (first dose administration)
- PLC < 100.000 platelets/mcL (at screening)
- Any clinical sign or known history of arterial thrombotic event or deep venous- thrombosis or pulmonary embolism
- HIV positive with current $CD4_+$ count of < 200/µL
- Liver Cirrhosis
- Factor VIII/IX immune tolerance induction regimen planned to occur during the trial

- Known hypersensitivity to the study medication
- Parallel participation in another experimental drug trial or another marketed drug trial that may affect the primary study endpoint.

Prior and Concomitant Therapy

The following limitations were included in the study protocol:

- Simultaneous use of prothrombin complex concentrates, activated or not – or – plasma, was not permitted.
- Concurrent treatment with rFXIII medications was not allowed.
- Experience with concomitant administration of anti-fibrinolytics is limited and concomitant treatment with these products should be avoided.

Screening and baseline evaluation

Subjects were screened for eligibility by the Investigator based on the inclusion and exclusion criteria and informed consent was obtained prior to performance of any study-specific procedure.

All subjects' demographics and medical history were documented.

Clinical, PK and Immunogenicity Assessment

Assessment during the PK phase

A total of 48 patients who met the required entry criteria were enrolled to be randomly assigned to receive two injections separated by a washout period of 3 days. Patients were centrally registered and randomised to receive in a 2x2 cross-over setting either a single dose of two for the following products:

(A): AryoSeven 90 µg/kg and (B): NovoSeven 90 µg/kg, or

(C): AryoSeven 270 µg/kg and (D): NovoSeven 270 µg/kg.

Patients had to be hospitalised at time of study medication administration and plasma sampling. Before any treatment, a blood sample was obtained in all patients for testing for immunogenicity.

As soon as laboratory (local lab) results for immunogenicity become available, patients were scheduled for two dosing visits (visits 2 and 3) that were separated by a washout period of 3 days. Patients in prophylaxis with NovoSeven and AryoSeven (Iran) were requested to stop prophylactic treatment 3 days before the first dose of study medication (visit 2). Patients were hospitalised at the time of study medication and plasma sampling (visit 2 and 3). At visit 2 and 3, patients received either AryoSeven or NovoSeven as single dose injections.

The Investigator and staff were blinded to study treatment.

Drugs were administered by slow intravenous injection (in about 1 min).

Blood samples were collected from each patient 10 min- prior to dose administration and at 10 min, 20 min, 1 h, 3 h, 5 h, 8 h, 12 h, 24 h and 30 h after study medication administration.

Plasma samples were divided in aliquots, labelled and thereafter immediately frozen and stored at -70°C/-80 C° until assayed by a central laboratory for PK and PD determinations. The central laboratory staff who determined the FVII levels were blinded to the treatment.

At visit 3, before AryoSeven or NovoSeven administration, blood samples of each patient were obtained for testing for immunogenicity by the local laboratory.

Clinical response/ Immunogenicity follow up (Phase B)

At the end of the PK/PD phase (visit 3), patients received a number of vials of AryoSeven to treat bleeding or as prophylaxis for the following 12 months. Treatment with AryoSeven during this follow-up phase was administered at home, with dose and duration of treatment based on the Investigator's decision, and iv. self-injection either by parents, caregiver or a support person.

Patients received AryoSeven 90 µg/kg for one or more doses until resolution of bleeding (the dose and duration of treatment were based on the Investigator's decision), and were assessed for clinical efficacy, monitoring of inhibiting antibody formation, and collection of safety data.

Adverse events (AEs) were monitored throughout the trial.

Assessment of biochemistry, haematology and coagulation-related parameters (Hb, HC, RBC, WBC, PLT, PT, aPTT, Fibrinogen, D-Dimer, AT, BUN, creatinine, uric acid, BIL, LDH, AST (SGOT), ALT (SGPT), Na, Ca, P, glucose, albumin) was performed at screening, pre-dosing at visit 2 and 3, and every 3 months in the immunogenicity phase.

Pharmacokinetic Variables

The primary PK parameter for PK similarity testing was the area under the plasma activity- time curve from time 0 to infinity (**AUC0-inf**) of the factor VII clotting activity.

The secondary PK endpoints included the PK parameters AUC0-tlast, Cmax, time to reach Cmax: tmax, t½, AUCextra (fraction of the total AUCinf that was derived by extrapolation beyond tlast), λz, CL, MRT and Vd were also tested.

PK assessment by measurement of plasma level of circulating factor VII was performed by the central lab, blinded to patients' treatment. Partial validation by the central lab was demonstrated for the commercial Staclot.

All PK primary and secondary parameters were determined by the central laboratory: European Research Biology Center (ERBC), Via Tito Speri 12, 00071 Pomezia (Roma), Italy.

Efficacy and Safety Variables

Patients were followed for assessment of efficacy, immunogenicity and evidence of adverse effects for 12 months. Clinical response in the treatment of bleeding during the immunogenicity phase was a secondary efficacy parameter.

Statistical Methods

Determination of Sample Size

The sample size estimate was based on the primary endpoint of area under the plasma concentration – time curve from time 0 to last quantifiable plasma concentration (AUClast) and maximum plasma concentration (Cmax). For the estimation of the coefficient of variation (CV%), Villar et al described that CV% was between 21% (adults) to 28% (children in the age of 2-12) of the AUClast parameter in earlier studies measuring FVII:C concentrations (Villar 2004). For the estimation of CV% in the present study for AUClast and Cmax, a CV% of 26% was assumed due to the following reasons:

1. Adolescents are included in this trial but not children. Less variation for adolescents than for children was assumed, but a higher variation than for adults. This lead to a value of approximately 26%, which was assumed as conservative estimation.

2. From 1, there was an expectation CV% for AUClast between 21% and 26%. Because Cmax tends to a lightly higher variation compared to AUClast, it was decided to take the upper value of that range (26%) for sample size estimation.

3. It was assumed that the variation was independent from the dose given.

Setting the type I error rate on the predefined level of one-sided 5% (two one sided tests with α=5% each) and assuming a coefficient of variation (CV) of 26% for the parameter AUClast and Cmax, 24 patients in a crossover setting were considered sufficient to show equivalence within [0.8; 1.25] with a power of 80% when the true ratio of test and reference is 1 (program PASS 11). Because 2 dosage groups (90 µg/kg, 270 µg/kg) have to be tested on bioequivalence in separate 2x2 crossover allocations within this trial, 24 patients need to be included in each crossover allocation, which sum-up to 48 patients in total.

A discrepancy is noted between the two primary endpoints described in the sample size calculations (AUC-last and Cmax) and the single primary endpoint defined in other sections of the latest protocol version (AUC-inf). It is assumed to be related to a change of primary endpoint following the 2016 Scientific Advice, with the sample size section of the protocol not being updated accordingly.

Analysis Sets

PK/PD analysis set (PAS)

The PK/PD analysis set (PAS) was to include all subjects who received all the study treatments which they were randomly assigned to, and do not have any major protocol deviations.

Safety evaluation set (SES)

The safety evaluation set (SES) was to include all subjects exposed to at least one dose of the study medication and having any documented safety data.

Immunogenicity analysis set (IAS)

The immunogenicity analysis set (IAS) was to include all subjects who have data from at least one visit after the PK/PD phase.

Individual data

The individual time courses of the plasma concentrations of FVII:C and PD parameter TGA, D-dimer and F1.2 were to be listed and presented graphically by subject and separately by treatment (AryoSeven 90 µg/kg, NovoSeven 90 µg/kg and AryoSeven 270 µg/kg, NovoSeven 270 µg/kg) using both linear and semi-logarithmic scale.

Summary data

For the descriptive statistics per time point of profiling, the planned sampling times were to be used. Summary statistics were planned be presented by treatment (Test: AryoSeven 90 µg/kg and 270 µg/kg /Reference: NovoSeven 90 µg/kg and 270 µg/kg) and time point of profiling, if at least 2/3 of the plasma concentrations were above LLOQ at the given time point.

The default summary statistics for plasma concentrations and PD parameter TGA, D-dimer and F.1.2 were the number of valid observations (N), number of missing observations (Nmiss), arithmetic mean, standard deviation (SD), coefficient of variation (CV), minimum (Min), median (MD), maximum (Max), geometric mean and the CV of the geometric mean.

Analysis of PK Parameters: General model assumptions

For the pharmacokinetic analyses based on FVII concentrations obtained from the FVII:C assay, the baseline FVII concentrations were be taken into account by subtracting the pre-dose concentrations from subsequent FVII concentrations to adjust for endogenous activated FVII (see also Villar et al. (40)). A non-compartmental PK method was planned to be used to analyze and summarise the individual time courses of plasma concentrations of FVII:C.

Analysis of primary PK parameter

The following primary PK parameter was planned to be calculated separately for

• AryoSeven 90 µg/kg with NovoSeven 90 µg/kg and

• AryoSeven 270 µg/kg with NovoSeven 270 µg/kg,

using a non-compartment model to obtain a further characterisation of the concentration-time curve of both eptacog alpha applications with respect to distribution and elimination of the drug:

AUCinf area under the plasma concentration-time curve from 0 to the infinity, based on the last observed concentration.

$$AUC_{\inf} = AUC_{0-tlast} + \frac{C_{last}}{\lambda_z},$$

First, the treatment effect (difference of values in period II – values in period I), the sequence effect (sum of values in period I and period II) and the period effect (difference of values in period with AryoSeven – NovoSeven) were summarised descriptively and presented by sequence, for each dose level separately. Second, the results of the ANOVA models (separately for each dose level), with fixed effects for treatment, sequence and period (treatment by sequence interaction) as well as a random effect to account for subjects within sequence were summarised and the carry-over, treatment and period effect presented.

Assessment of biosimilarity was then to be based upon 90% confidence intervals for the ratio of the population geometric means (test: AryoSeven/ reference: NovoSeven), for each dose level, i.e. 90 µg/kg and 270 µg/kg.

The data was transformed prior to analysis using a logarithmic transformation. A 90% confidence interval (CI) for the difference in AUCinf-values between both treatments on the log-transformed scale was also obtained from the ANOVA model. The resulting confidence interval was then back transformed to obtain the 90% confidence interval for the ratio on the original scale.

Analysis of secondary PK parameters

The following secondary PK endpoints were evaluated analogously to the primary PK endpoint:

AUClast, AUCextra, λz, CL, Vd, AUCinf-norm, AUClast-norm, Cmax, Cmax-norm

The continuous parameters MRT and $t_{1/2}$ were analyzed using a similar ANOVA and confidence interval, but without logarithmic transformation and retransformation.

Analysis of primary PD parameters

Each PD endpoint was planned to be analysed in the same way as the primary PK endpoint. Thus first, the treatment effect (difference of values in period II – values in period I), the sequence effect (sum of values in period I and period II) and the period effect (difference of values in period with AryoSeven – NovoSeven) have been summarised descriptively for each characteristic, and presented by sequence.

Second, the results of the ANOVA models (separately for each dose level), with fixed effects for treatment, sequence and period (treatment by sequence interaction) as well as a random effect to account for subjects within sequence were to be summarised and the carry-over, treatment and period effect presented.

Assessment of biosimilarity was then to be based upon the 90% confidence intervals for the ratio of the population geometric means (test: AryoSeven/ reference: NovoSeven), for each dose level, i.e. 90

µg/kg and 270 µg/kg as it was done for the primary PK endpoint. The data of the PD endpoints were also be transformed prior to analysis using a logarithmic transformation.

A strong evidence of biosimilarity was supposed to be given, if the CIs were completely contained within the corresponding biosimilarity margin PD_{marg} for both dose levels, and each PD endpoint.

Instead of using the 80/125 rule as it was done for the PK endpoint, a linear scaled margin was planned to be determined to take the possible high within-subject-variability of the respective PD parameters into account. Since this study has been planned as a cross-over design and not sufficient information was available on the variability of the parameters of interest, it was decided on following Zhang et al.to justify widening of acceptance ranges.

The fixed cutoff linear scaled margin is described as (Chow 2016)

$$PD_{marg} = \begin{cases} \theta_0, & if\ CV_p \leq CV_0 \\ \frac{CV_p}{CV_0} \times \theta_0, & if\ CV_p > CV_0 \end{cases},$$

where θ_0 is a fixed margin commonly accepted for investigating bioequivalence or biosimilarity, here the originally planned margin of the 80/125 rule was planned to be used.

CVp is the coefficient of variation of the PD endpoint of interest determined as

$$CV_p = \frac{\sqrt{MSE}}{\overline{X_R}},$$

where MSE is the mean square error (an estimate for the within subject variability) and X_R is the least square mean for the reference (i.e. NovoSeven), both can be taken from the ANOVA model.

And CV_0 is a chosen cutoff for high-variability drug definition, set to 30%.

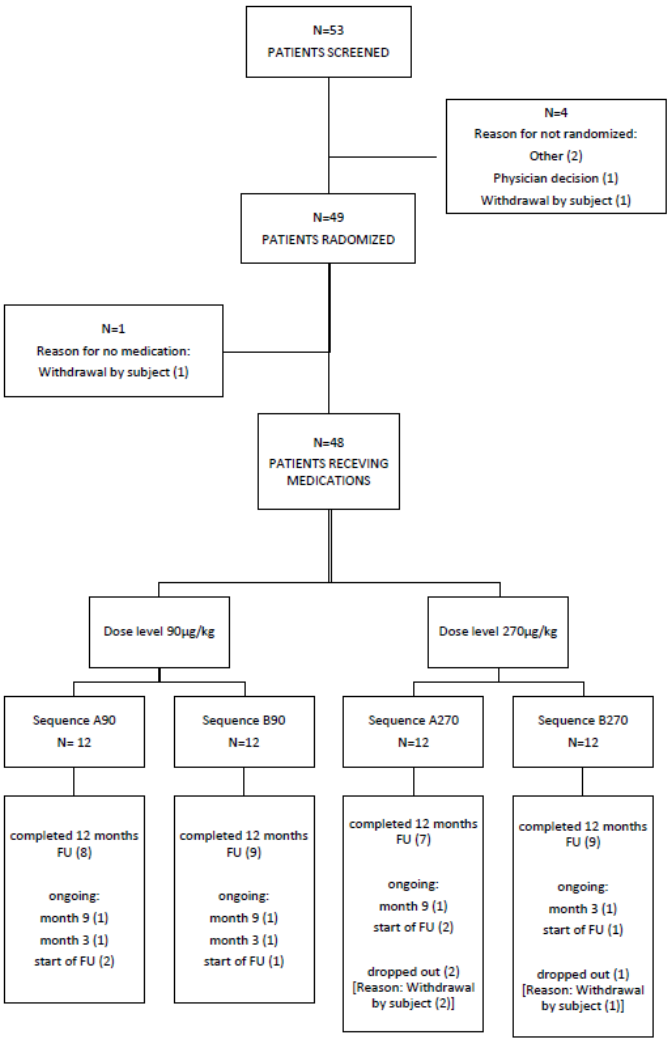
Analysis of safety data

Adverse Events were coded by MedDRA version 23.1. During the study, all AEs and Medical History were coded using MedDRA version 19. However, before statistical analysis, all AE and Medical History were re-coded (by the Statisticians) to MedDRA version 23.1. All AEs were summarised by MedDRA body system and preferred term.

Immunogenicity data from the central lab have been used for the statistical analyses to minimise errors and results variability. In the immunogenicity period, the number of patients with one or more bleedings and - thereof – the percentage of patients who developed treatment related antibodies to Factor VII were to be tabulated for each 3-month period and overall (over the complete 12 month period). If immune reactions were observed, a relationship to the number of treated bleedings within the respective period had to be evaluated.

Results

Participant flow



From the overall 53 screened subjects, 49 were randomised and 48 subjects received at least one dose of study drug. The study population consisted of 48 patients with haemophilia A/B with inhibitors. According to the CSR, the database was frozen for statistical analysis, so 12 patients have not yet been completed (and is ongoing for secondary endpoint assessment).

Conduct of the study

The original protocol was dated 28 November 2016 and was amended two times: 5 October 2018 (in Turkey it was dated 5 March 2019), and 19 March 2020 (in Turkey 3 June 2020). First patient was enrolled on 17 September 2018.

Protocol Amendment #1 (dated 5 October 2018) included the following key changes:

- The wash-out period has been reduced from 2 weeks to 3 days.
- Treatment of bleeding at home was allowed.
- Prophylaxis with AryoSeven was allowed.
- Criteria for evaluation of response to the treatment of bleeding at home have been included (4 point scale: Excellent, Good, Moderate, None).

- Plasma sampling for immunogenicity was changed to collection every 3 months and persist in the absence of response to AryoSeven.
- A procedure for providing to the patients with a supply of AryoSeven to use at home for treatment of bleeding or prophylaxis has been added. Study drug accountability of the drug supplied to the patient for treatment of bleeding at home or prophylaxis has been specified.
- Text change of the analytical method for measurement of FVII clotting activity.
- Randomisation is performed using an IWRS system and it has been stratified by Country and not by the centre as stated in the original protocol.
- The use of the prothrombin complex concentrate FEIBA was allowed, but due to its half-life, patients receiving FEIBA must stop treatment at least 2 weeks before the start of the study.
- The Central Laboratory for efficacy and safety assessments has been selected.
- A new revised and complete procedure for protocol deviations has been included.
- The criteria for evaluation of response to the treatment of bleeding at home has been added for clarity. Additional descriptive analyses have been added.
- Comparison of the immunogenicity pattern after a single dose of AryoSeven in comparison to NovoSeven (i.e. the first dose of drug in the cross-over phase) has been removed.
- An eCase report form is used in the study and a description has been included.

Protocol Amendment #2 (dated 19 March 2020) was mainly administrative.

Refusals in the study protocol: Two sections (5.3.1 and 9.1) include erroneously the "maximum plasma activity Cmax" as a primary endpoint. However, the correct definition of Primary Pharmacokinetic endpoint is AUCinf.

Protocol violation was recorded for 34.7% of the subjects, which were classified as minor.

Data Sets Analysed

All 48 subjects completed the PK/PD cross-over phase and were included in the PK/PD analysis set (PAS). These patients entered also the immunogenicity follow-up phase. Three subjects discontinued during the follow-up phase [for a total of 4 subjects discontinued (8.2%)] due to withdrawal by subject. Overall, at the time of database freezing for the statistical analysis, 33 subjects completed the 12 months immunogenicity phase. The remaining 12 subjects were ongoing during this assessment.

Table 1. Summary of subject disposition by sequence

Disposition of patients	Sequence A90 (N=12)	Sequence B90 (N=12)	Sequence A270 (N=13)	Sequence B270 (N=12)	Total (N=53)
Subjects Screened	-	-	-	-	53
Subjects not randomised	-	-	-	-	4
Subjects Randomised	12 (100%)	12 (100%)	13 (100%)	12 (100%)	49 (100%)
Subjects with protocol deviations	3 (25%)	4 (33.3%)	5 (38.5%)	5 (41.7%)	17 (34.7%)
Subjects with major protocol violations	-	-	-	-	-
Subjects completed PK/PD phase	12 (100%)	12 (100%)	12 (92.3%)	12 (100%)	48 (98.0%)
Subjects discontinued the study [1]	-	-	3 (23.1%)	1 (8.3%)	4 (8.2%)
Safety Population (SES)	12 (100%)	12 (100%)	12 (92.3%)	12 (100%)	48 (98.0%)
PK Population (PAS)	12 (100%)	12 (100%)	12 (92.3%)	12 (100%)	48 (98.0%)
Immunogenicity Population (IAS) [2]	10 (83.3%)	11 (91.7%)	10 (76.9%)	11 (91.7%)	42 (85.7%)

Sequence A90: Starting with AryoSeven 90 µg/kg followed by NovoSeven 90 µg/kg
Sequence B90: Starting with NovoSeven 90 µg/kg followed by AryoSeven 90 µg/kg
Sequence A270: Starting with AryoSeven 270 µg/kg followed by NovoSeven 270 µg/kg
Sequence B270: Starting with NovoSeven 270 µg/kg followed by AryoSeven 270 µg/kg

[1] Three subjects who discontinued the study during the immunogenicity follow-up (1004010, after V3; 100445, after V3; 200301, after M+3 visit); One subject discontinued after randomization and did not receive treatment (500101).

[2] The study is still ongoing, so data on the immunogenicity follow-up is not complete. Six patients were at beginning of the follow-up (100467, 100468, 100469, 100605, 100606, 100607) and consequently data on bleeding response are available for 42 patients (see Figure 10-1 for subject disposition).

Table 2. Patient Demographic and Baseline Characteristics

Table 14.1.2.2 Demographic and Baseline Characteristics
Population: PK/PD Analysis Set
Final Analysis (PK/PD Phase)

	Sequence A90 (N=12)	Sequence B90 (N=12)	Sequence A270 (N=12)	Sequence B270 (N=12)	Total (N=48)
Age [years]					
n	12	12	12	12	48
Mean	24.4	22.8	32.7	30.5	27.6
Std	7.83	10.11	11.28	10.18	10.47
Median	24.5	19.5	30.0	32.0	27.5
Q1 - Q3	18.0 - 28.5	14.0 - 30.5	23.5 - 40.5	22.0 - 38.5	19.0 - 35.0
Min - Max	14 - 40	12 - 42	20 - 56	15 - 46	12 - 56
Age [years], categorised					
< 18 years	3 (25.0%)	5 (41.7%)	-	3 (25.0%)	11 (22.9%)
>= 18 years	9 (75.0%)	7 (58.3%)	12 (100%)	9 (75.0%)	37 (77.1%)
n	12	12	12	12	48
Sex					
Male	12 (100%)	12 (100%)	12 (100%)	12 (100%)	48 (100%)
n	12	12	12	12	48
Race					
White	12 (100%)	12 (100%)	12 (100%)	12 (100%)	48 (100%)
n	12	12	12	12	48

Height [cm]					
n	12	11	12	12	47
Mean	172.8	168.6	174.3	176.3	173.1
Std	8.20	8.05	7.01	6.66	7.76
Median	171.5	170.0	176.0	176.0	173.0
Q1 - Q3	167.5 - 176.0	160.0 - 176.0	170.5 - 179.0	171.0 - 181.0	168.0 - 179.0
Min - Max	161 - 193	158 - 180	158 - 183	166 - 187	158 - 193
Weight [kg]					
n	12	12	12	12	48
Mean	65.90	59.99	66.21	64.54	64.16
Std	13.976	14.271	15.355	14.376	14.257
Median	65.95	60.05	65.20	62.15	62.60
Q1 - Q3	53.45 - 72.80	51.73 - 73.25	50.10 - 79.85	52.23 - 79.50	52.20 - 76.50
Min - Max	45.9 - 91.8	35.0 - 79.5	47.0 - 89.7	43.5 - 86.0	35.0 - 91.8

Note: N: number of subjects in a sequence group; n: number of subjects; %: percentage based on N
Mean=arithmetic mean, Std=standard deviation, Q1/Q3=1st/3rd quartile, Min=minimum, Max=maximum
Sequence A90: Starting with AryoSeven 90 µg/kg followed by NovoSeven 90 µg/kg
Sequence B90: Starting with NovoSeven 90 µg/kg followed by AryoSeven 90 µg/kg
Sequence A270: Starting with AryoSeven 270 µg/kg followed by NovoSeven 270 µg/kg
Sequence B270: Starting with NovoSeven 270 µg/kg followed by AryoSeven 270 µg/kg

In total, the mean number of bleeding (SD) events during the last 12 months was 22.3±24.97. In 31 patients (64.6%) the cause for the last/most recent bleeding episode was spontaneous and in 17 patients (35.4%) the cause was traumatic. In 18 patients (37.5%) the last/most recent bleeding episode was treated with AryoSeven, and 19 patients (39.6%) received FEIBA. The history of bleeding episodes and corresponding former treatment were similar between the treatment arms that received the higher dose of 270 µg/kg (A270 and B270). At the lower dose of 90 µg/kg, bleeding history was unbalanced, with patients in sequence A90 having a lower number of bleeds during the previous 12 months.

Patients were balanced with regard to the frequency of pre-existing disorders.

Pharmacokinetics Analysis

Of the 49 (100%) patients randomised, 48 (98.0%) completed the PK/PD phase and were included in the PAS.

Table 3. Primary endpoint

Table 11-7. Bioequivalence Assessment of Primary PK endpoint AUC_{inf} (PK/PD Analysis Set)

	Dose Level	Statistical Method	Point estimate	Confidence limits		Level of confidence
				lower	upper	
AUC_{inf}	90	ANOVA	0.9995	0.9805	1.019	0.9
	90	Two one-sided t-test	0.9995	0.9772	1.0223	
	270	ANOVA	0.9631	0.917	1.0116	
	270	Two one-sided t-test	0.9623	0.9078	1.0202	

The 90 µg/kg iv. dose and the 270 µg/kg iv. of HemAryo and EU NovoSeven were both considered bioequivalent in terms of the primary endpoint AUCinf as the 90% CI for the geometric LS means ratios were fully contained within the predefined bioequivalence limits of 0.80 to 1.25.

Secondary endpoints

Table 11-11. Secondary Pharmacokinetic Analysis: Bioequivalence assessment (PK/PD Analysis Set)

Parameter	Dose level	Statistical Method	Point estimate	Confidence limits		Level of confidence
				lower	upper	
AUC_{extra} [%]	90	ANOVA	0.9606	0.6143	1.5023	0.9
	270	ANOVA	0.7667	0.5292	1.1108	
$AUC_{inf\text{-}norm}$ [h*IU/mL]	90	ANOVA	1.0014	0.9823	1.0209	0.9
	270	ANOVA	0.9631	0.9169	1.0115	
AUC_{last}	90	ANOVA	0.9772	0.9384	1.0177	0.9
	270	ANOVA	0.9687	0.9235	1.0162	
$AUC_{last\text{-}norm}$ [h*IU/mL]	90	ANOVA	0.9786	0.9395	1.0193	0.9
	270	ANOVA	0.9686	0.9234	1.0161	
CL_{sys} [mL/h]	90	ANOVA	0.9986	0.9795	1.018	0.9
	270	ANOVA	1.0384	0.9886	1.0906	
C_{max} [IU/mL]	90	ANOVA	0.9868	0.9593	1.0152	0.9
	270	ANOVA	0.9805	0.9347	1.0286	
$C_{max\text{-}norm}$ [IU/mL]	90	ANOVA	0.9882	0.9603	1.0169	0.9
	270	ANOVA	0.9804	0.9346	1.0285	
Lambda [h^-1]	90	ANOVA	0.9564	0.8868	1.0314	0.9
	270	ANOVA	1.0323	0.9991	1.0667	
MRT [h]	90	ANOVA	0.0314	-0.0884	0.1513	0.9
	270	ANOVA	-0.0523	-0.0916	-0.013	
Vd [mL]	90	ANOVA	1.0441	0.9613	1.1341	0.9
	270	ANOVA	1.0058	0.9476	1.0677	
$t_{1/2}$ [h]	90	ANOVA	0.1243	-0.0526	0.3012	0.9
	270	ANOVA	-0.0796	-0.1612	0.0021	
t_{max} [h]	90	Wilcoxon*	0	0	0	0.9
	270	Wilcoxon*	0	-0.008333	0	

* Wilcoxon signed rank test; Source: Table 14.2.4.3

The 90% CIs were within the equivalence margin for the parameters AUCinf-norm, AUClast, AUClast-norm, CLsys, Cmax, Cmax-norm, Lambda, Vd for both doses (90 µg/kg and 270 µg/kg). However, there was unexpectedly high variability observed for AUCextra, resulting in 90% CIs broader than expected. The 90% CI for AUCextra was (0.6143–1.5023) for the 90 µg/kg dose and (0.5292–1.1108) for the 270 µg/kg dose.

Overlay of plasma concentration time curves

For AryoSeven and the reference product NovoSeven, one patient who received a dose of 90 µg/kg (subject ID: 200401; sequence A90; the corresponding individual concentration-time curves are shown below) had particularly high concentration values at the time point 10 minutes after administration compared to all other patients, where e.g., concentration values ranged from 50 to 100 IU/ml. In contrast, the Cmax achieved in this patient were about 150 IU/ml and 200 IU/ml after treatment with AryoSeven and NovoSeven, respectively.

Figure 2

Figure 14.4.2.1 FVII clotting activity – Overlay of Plasma Concentration after Administration of 90 µg/kg AryoSeven (Linear Scale)
Population: PK/PD Analysis Set
Final Analysis (PK/PD Phase)

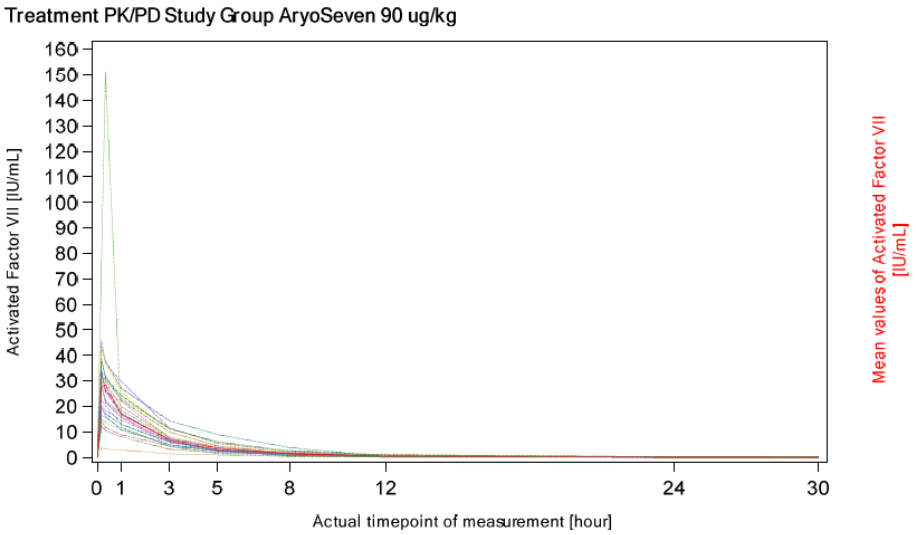
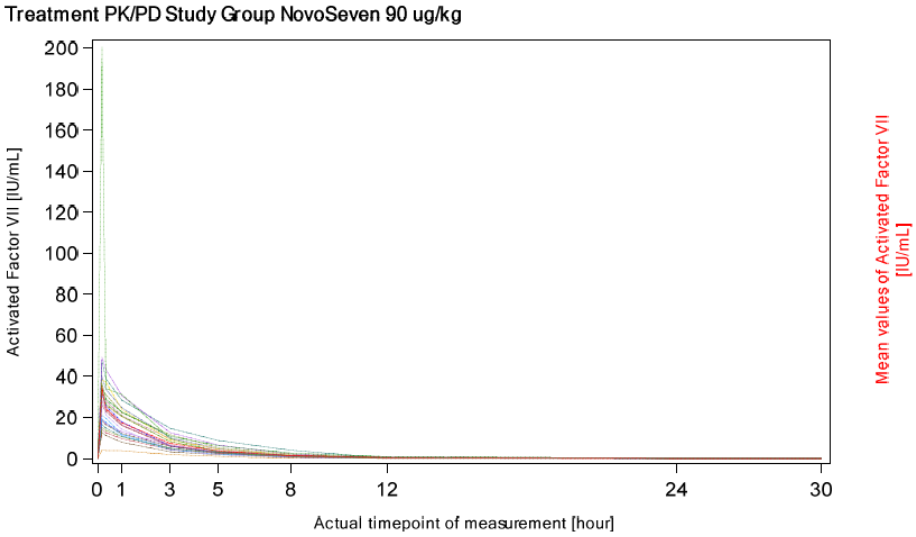


Figure 3

Figure 14.4.2.2 FVII clotting activity – Overlay of Plasma Concentration after Administration of 90 µg/kg NovoSeven (Linear Scale)
Population: PK/PD Analysis Set
Final Analysis (PK/PD Phase)

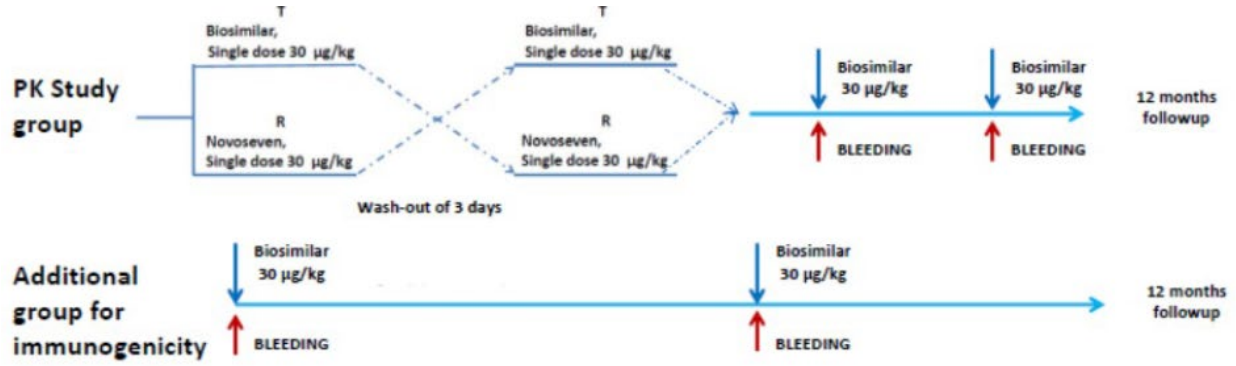


UGA 2014-02

Methods

Study UGA 2014-02 was a multicentre, randomised, single dose, double blinded, two-way cross-over study, evaluating the pharmacokinetic of biosimilar eptacog alfa (AryoSeven) and NovoSeven (Novo Nordisk) in adult and paediatric (>12 years) patients with a confirmed diagnosis of severe congenital Factor VII Deficiency (FVII <1%), with >2 episodes of bleeding/year requiring treatment with FVII infusions.

Figure 4: Study design and schematic presentation of treatment allocation



The primary objective was to demonstrate similar pharmacokinetic profile of the biosimilar eptacog alfa (AryoSeven) and NovoSeven with regards to key PK parameters.

Secondary objectives included:

- To demonstrate similar pharmacokinetic profile of the biosimilar eptacog alfa (AryoSeven) and NovoSeven with regards to other PK parameters;
- To evaluate clinical response in controlling acute bleeding;
- Immunogenicity;
- Adverse events.

The protocol planned for 24 subjects, not bleeding, to be enrolled and randomised to receive either a single dose of AryoSeven 30 µg/kg and one single dose of NovoSeven 30 µg/kg, or vice versa, with doses separated by a washout period of 3 days.

Subjects in routine prophylaxis with Novoseven or AryoSeven (in Iran) had to stop prophylactic treatment 3 days before starting the trial (when they received the first dose of study medication). Subjects had to be hospitalised at time of study medication administration and blood sampling.

At the end of PK phase, all subjects were followed to receive AryoSeven 30 µg/kg on demand for every bleeding episode that occurred during the following 12 months or prophylaxis with AryoSeven, with the aim of monitoring of inhibiting antibody formation, lack of efficacy and collection of safety data. Dose, frequency and duration of treatment should be modified based on the Investigator's decision.

An additional group of 26 subjects with a confirmed diagnosis of severe Factor VII Deficiency (FVII <1%) was planned for immunogenicity assessment. These subjects would not enter the PK phase of the study but they were to be followed to receive AryoSeven 30 µg/kg on demand, for treatment of bleeding or prophylaxis of bleeding, for 12 months, with the same modality of the follow-up for immunogenicity of the PK group. The aim was the monitoring of inhibiting antibody formation, lack of efficacy and collection of safety data.

Inclusion and exclusion criteria

- Patients with a confirmed diagnosis of congenital, severe Factor VII deficiency (FVII <1%), with > 2 episodes of bleeding/year requiring treatment with FVII infusions, in non-bleeding

status. Patients for the Additional group for immunogenicity should be enrolled when a bleeding episode occurred, requiring treatment with FVII.

- Male and female subjects
- Adult and children (>12 years)
- Patients must also provide voluntary written informed consent to the protocol to be eligible for the study. For minor patients, parent/legal guardian had to provide consent and, when possible, patient assent had also been obtained. For compromised patients, their designated proxy must have provided informed consent.

Patients in the Additional Group for Immunogenicity should have any type of bleeding (major bleeding and minor bleeding) requiring treatment with rFVIIa (according to the Investigator decision).

Patients who fulfilled any of the following criteria were not enrolled:

- Any other type of congenital or acquired coagulopathy (except congenital Factor VII deficiency), such as: liver disease (hepatitis), vitamin K deficiency, uremia, malignancy.
- Antibodies against Factor VII
- Patients entering the PK Study Group who have not suspended prophylactic regimen with Novoseven or AryoSeven 3 days before starting the trial (receiving first dose of study medication).
- Patients entering the Additional Group for Immunogenicity study, only, who have been exposed to AryoSeven before starting study. [Patients who have received Novoseven (on demand or in prophylaxis) before starting study were allowed.]
- Platelet count less than 100.000 platelets/µl (at screening visit)
- Patients who have received routine (prophylactic) treatment with rFVIIa in the period between screening visit (visit 1) and visit 2 of this study (first dose administration)
- Any clinical sign or known history of arterial thrombotic event or deep venous- thrombosis or pulmonary embolism
- HIV positive with current CD4+ count of less than 200/µl
- Liver cirrhosis
- Known hypersensitivity to the study medication
- Parallel participation in another experimental drug trial.
- Parallel participation in another marketed drug trial that may affect the primary end point of the study.

Prior and Concomitant Therapy

The following limitations were included in the study protocol:

- Simultaneous use of prothrombin complex concentrates, activated or not – or – plasma, was not permitted.
- Concurrent treatment with rFXIII medications was not allowed.
- Experience with concomitant administration of anti-fibrinolytics is limited and concomitant treatment with these products should be avoided.

Screening and baseline evaluation

Subjects were screened for eligibility by the Investigator based on the inclusion and exclusion criteria and informed consent was obtained prior to performance of any study-specific procedure.

All subjects' demographics and medical history were documented.

Clinical, PK and Immunogenicity Assessment

Assessment during the PK phase

Patients were scheduled for two dosing visits (visits 2 and 3) separated by a washout period of 3 days. Patients in prophylaxis with Novoseven and AryoSeven (Iran) were requested to stop prophylactic treatment 3 days before visit 2 (first dose of study medication). Patients had to be hospitalised at the time of study medication administration and plasma sampling (visit 2 and 3).

At visit 2 and 3, patients received study medications (AryoSeven or NovoSeven single dose injection). The investigator and staff and all laboratory personnel were blinded to study treatment. Blood samples for the PK study were collected from each patient 10 min- prior to dose administration and at 10 min, 20 min, 1 h, 3 h, 6 h, 8 h, 12 h, 24 h and 30 h after study medication administration. Blood samples (5 ml) were centrifuged within max 1 hour of collection at 2500 g (room temperature) for 15 min. Plasma samples were divided in aliquots, labeled and thereafter immediately frozen and stored at -70°C/-80°C until assayed by a central laboratory.

Factor VII activity levels in the plasma samples were determined by FVII-rTF assay, (commercial Staclot® VIIa–recombinant tissue factor assay, Diagnostica Stago, Asniéres sur Seine, France). The FVII plasma concentrations were measured using a validated bioanalytical method, and according to ERBC (the central lab) SOPs, EMA and FDA Guidelines.

Assessment during the Immunogenicity phase

At visit 3, before AryoSeven or NovoSeven administration, for each patient, blood samples were obtained for testing for immunogenicity (assessment of neutralising antibodies against eptacog alfa) by the local laboratory.

At visit 3 (end of the PK phase), patients received a number of vials of AryoSeven enough for treatment of bleeding or for prophylaxis for the following 3 months. The number of vials was decided by the Investigator, based on the dose prescribed for the treatment of each bleeding, and number of episodes of bleeding in the 3 months before entering the study – or – on dosage and frequency of administration for prophylaxis. At following every 3 months visits, patients received additional vials. Patients were trained by the centre personnel on how to report the results of the treatment of bleeding, according to the criteria set for this study.

During the 12 months follow-up period, patients:

- received treatment with AryoSeven 30 µg/kg (for one or more days until resolution of bleeding) for every bleeding episode that should occur during 12 months – or – for prophylaxis with AryoSeven, with dose and frequency that should be modified based on Investigator's decision, with the aim of monitoring of inhibiting antibody formation, lack of efficacy and collection of safety data.
- had to return to the centre every 3 months for 1 year (4 visits) for blood sampling for immunogenicity, and adverse event monitoring (including laboratory).

Immunogenicity testing was done also when patient show lack of efficacy to the treatment with AryoSeven. All immunogenicity testing were performed by the local laboratory and samples were shipped to the central lab for confirmation.

Adverse events (AEs) were monitored throughout the trial.

Assessment of biochemistry, haematology and coagulation-related parameters (Hb, HC, RBC, WBC, PLT, PT, aPTT, Fibrinogen, D-Dimer, AT, BUN, creatinine, uric acid, BIL, LDH, AST (SGOT), ALT (SGPT), Na, Ca, P, glucose, albumin) was performed at screening, pre-dosing at visit 2 and 3, and every 3 months in the immunogenicity phase.

An End-of-Treatment assessment was arranged for subjects who were removed from the study due to subject request or treatment related toxicities.

Randomisation and Blinding

Randomisation was planned to be performed in a 1:1 manner using an IWRS (Interactive Web Response System).

Blinding was planned to be performed by an independent third-party operator (nurse/pharmacist, unblinded), who have prepared undistinguishable syringes with subject's dosing and labelling.

Treatment

All patients enrolled in the PK phase received either a single dose of AryoSeven 30 µg/kg and one single dose of NovoSeven 30 µg/kg, or vice versa (based on the randomisation list), with doses separated by a washout period of 3 days. Both drugs were administered by slow intravenous injection.

In the event that a patient had a bleeding episode between the two doses, during the PK cross over phase, he/she was treated with AryoSeven, with dose and duration set on investigator decision, until resolution of bleeding. The second dose/product of the PK cross over phase was postponed and administered after 3 days from the end of the treatment for bleeding.

At the end of the PK phase, all patients received AryoSeven 30 µg/kg on demand, for every bleeding episode that occurred during 12 months [AryoSeven was administered every 4-6 hours, for one or more doses until haemostasis was achieved (resolution of bleeding)] – or – prophylaxis with AryoSeven. Dose, frequency and duration of treatment should be modified based on the Investigator's decision. The modality of treatment with AryoSeven (on demand at study centre or home, or prophylaxis) was decided by the Investigator.

Pharmacokinetic Variables

Pharmacokinetic assessment by measurement of plasma level of circulating factor VII using a clotting method, the commercial Staclot VIIa–recombinant tissue factor assay (Diagnostica Stago, Asniéres sur Seine, France) performed by the central lab, blinded to patients' treatment. Partial validation by the central lab was demonstrated for the commercial Staclot.

All PK primary and secondary parameters were determined by the central laboratory: EUROPEAN RESEARCH BIOLOGY CENTER (ERBC), Via Tito Speri 12, 00071 Pomezia (Roma), Italy. The FVII plasma concentrations were measured using a validated bioanalytical method, and according to ERBC SOPs, EMA and FDA Guidelines.

Primary PK parameters were the area under the plasma activity-time curve from time 0 to last quantifiable activity (AUC_{last}) and maximum plasma concentration of the factor VII clotting activity, C_{max}.

Secondary PK parameters included comparison of:

- Area under the plasma activity-time curve from time 0 to infinity: AUC_{inf},
- Time to reach C_{max}: t_{max}
- Terminal elimination half-life: t½,
- Fraction of the total AUC_{inf} that was derived by extrapolation beyond t_{last}: AUC_{extra}
- First order rate constant associated with the terminal (log-linear) portion of the curve: λz
- Total plasma clearance: CL,
- Mean residence time: MRT
- Volume of distribution: V_d

Efficacy and Safety Variables

Patients were followed for assessment of efficacy, immunogenicity and evidence of adverse effects for 12 months. Clinical response in the treatment of bleeding during the immunogenicity phase was a secondary efficacy parameter.

Statistical Methods

Determination of Sample Size

The sample size estimate was based on the primary endpoint of area under the plasma activity-time curve from time 0 to last quantifiable plasma concentration (AUClast) and maximum plasma concentration (Cmax). For the estimation of the coefficient of variation (CV%), Villar 2004 described that CV% was between 21% (adults) to 28% (children in the age of 2-12) of the AUClast parameter in earlier studies measuring FVII:C concentrations.

For the estimation of CV% in the present study for AUClast and Cmax, a CV% of 26% was assumed.

Setting the type I error rate on the predefined level of one-sided 5% (two one sided tests with α=5% each) and assuming a coefficient of variation (CV) of 26% for the parameter AUClast and Cmax, 24 patients in a crossover setting were considered sufficient to show equivalence within [0.8; 1.25] with a power of 80% when the true ratio of test and reference is 1 (program PASS 11).

Analysis Sets

The PK analysis set (PAS) included subjects who:

- received both the study treatments to which they were randomly assigned, and
- did not have concentrations of FVII ≥1 IU/ml before single dose administration, and
- did not have any major protocol deviations, including a deviation of sampling time at the end of the infusion (i.e. provided an end of infusion sample and at least 12 hours after study drug administration).

Protocol violations or deviations were any non-adherences to the procedures outlined in the study protocol and include, but were not limited to, late evidence of exclusion criteria, missed evaluations, incorrect timing of evaluations or dosing and intake of prohibited medication.

The safety evaluation set (SES) included all patients exposed to at least one dose of the study medication and any documented safety data.

Subject Disposition

The number of subjects screened and randomised, the number of subjects in each analysis population, the number of subjects who have major protocol deviations, and the number of subjects who completed/discontinued were summarised using frequencies and percentages for all subjects.

Pharmacokinetic Data Analysis

Individual data

The individual plasma concentration of AryoSeven/NovoSeven, including date/time, were to be listed. Individual concentrations reported by the laboratory as below the limit of quantification were to be presented as '<LOQ' in the tabulations and listings. Individual concentration-time curves were to be presented on linear and log-linear scale for all subjects in the PK set (PAS).

Summary data

For the descriptive statistics per time point of profiling, the planned sampling times were planned to be used. Summary statistics were to be presented by treatment (Test: AryoSeven/Reference: NovoSeven) and time point of profiling, if at least 2/3 of the plasma concentrations were above LLOQ at the given time point.

The default summary statistics for plasma concentrations were the number of valid observations (N), number of missing observations (Nmiss), arithmetic mean, standard deviation (Std), coefficient of variation (CV), minimum (Min), median (MD), maximum (Max), geometric mean and the CV of the geometric mean.

Plots of arithmetic mean and geometric mean time courses were to be provided separately by treatment using linear and semi-logarithmic scale. Summary statistics of plasma concentrations were to be calculated for each profiling time point by each treatment (AryoSeven/NovoSeven).

General model assumptions

For the pharmacokinetic analyses based on FVII concentrations obtained from the FVII assay, the baseline FVII concentrations were taken into account by subtracting the pre-dose concentrations from subsequent FVII concentrations to adjust for endogenous activated FVII (see also Villar 2004).

A non-compartmental PK method was to be used to analyze and summarise the individual time courses of plasma concentrations of FVII in accordance with following conventions:

- The individual time courses of the plasma concentrations has been summarised by means of a non-compartmental analysis.
- The actual sampling times has been used to fit the pharmacokinetic parameters.
- If the pre-dose concentration was greater than 5% of the individual's C_{max}, the subject should be dropped from all pharmacokinetic evaluations (see definition of PAS cohort above).
- The AUC parameters have been calculated according to the linear up/log down trapezoidal method, using the linear trapezoidal rule any time that the concentration data is increasing, and the logarithmic trapezoid rule is used any time that the concentration data is decreasing.
- If Cmax was not unique, the first maximum was used.
- λz has been estimated by log-linear least squares regression of the terminal part of the plasma concentration versus time curve.

The fixed effects associated with each subject in each period in each of the two groups were calculated as follows:

Group	Period 1	Period 2

Sequence A (AryoSeven – NovoSeven) $\mu + \pi 1 + \tau A$ryoSeven $\mu + \pi 2 + \tau N$ovoSeven + λA

Sequence B (NovoSeven – AryoSeven) $\mu + \pi 1 + \tau$NovoSeven $\mu + \pi 2 + \tau A$ryoSeven + λB

where the direct treatment effects of AryoSeven and NovoSeven respectively. $\pi 1$ and $\pi 2$ are the period effects of period 1 and 2. λA and λB are the corresponding carry-over effects for groups A and B respectively. μ is a general mean.

Primary Pharmacokinetic Analyses

The following primary single-dose pharmacokinetic parameters were to be derived from the time courses of the serum plasma concentrations of FVII activity using the non-compartment model in order to show the bioequivalence between eptacog alfa of AryoSeven (Test) and NovoSeven of Novo Nordisk (Reference) by a parametric statistical approach:

- AUC_{last}: area under the plasma concentration-time curve from time 0 to the last observed (and quantifiable) time point tlast
- C_{max}: observed maximum plasma concentration

Assessment of bioequivalence was based upon 90% confidence intervals for the ratio of the population geometric means (test/reference) for the parameters AUC_{last} and C_{max}. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level. The primary endpoint pharmacokinetic parameters AUC_{last} and C_{max} were analyzed using ANOVA. The data were transformed prior to analysis using a logarithmic transformation. A 90% confidence interval (CI) for the difference between formulations on the log-transformed scale is obtained from the ANOVA model. This confidence interval was then back-transformed to obtain the 90% confidence interval for the ratio on the original scale. If the CI was completely contained within the bioequivalence interval (0.80– 1.25), the two formulations were to be considered bioequivalent.

Secondary Pharmacokinetic Analyses

The following secondary PK parameters have been calculated using a non-compartment model to obtain a further characterisation of the concentration-time curve of both eptacog alpha applications with respect to distribution and elimination of the drug:

AUC_{inf}	area under the plasma concentration-time curve from time 0 to infinity, based on the last observed concentration
C_{last}	$AUC_{last}+C_{last}/\lambda z$
t_{max}	time of C_{max}
AUC_{extra}	fraction of the total AUC_{inf} that was derived by extrapolation beyond t_{last}
λz	First order rate constant associated with the terminal (log-linear) portion of the curve
t½	elimination half-life; calculated as ln(2)/λz
MRT	mean residence time
CL	Clearance
Vd	Volume of distribution

AUC, C_{max} and CL have also been evaluated dose normalised as secondary PK parameters ($AUC_{last\text{-}norm}$, $AUC_{inf\text{-}norm}$, $C_{max\text{-}norm}$, and CL_{norm}). This has been done for each patient by dividing the respective parameter though the individual dose given.

The kinetic parameter AUC_{inf}, and the dose-normalised parameters were evaluated descriptively analogously to the primary parameter. The continuous "Time-to-event" parameters MRT and t½ were calculated using a similar ANOVA and confidence interval as described above for primary analysis, but without logarithmic transformation and retransformation.

The test on differences between Eptacog alfa of AryoSeven and NovoSeven with regard to the categorical pharmacokinetic parameter tmax was to calculated using the Wilcoxon signed rank test and the Hodges Lehmann point estimator with the respective 90% confidence intervals.

Results

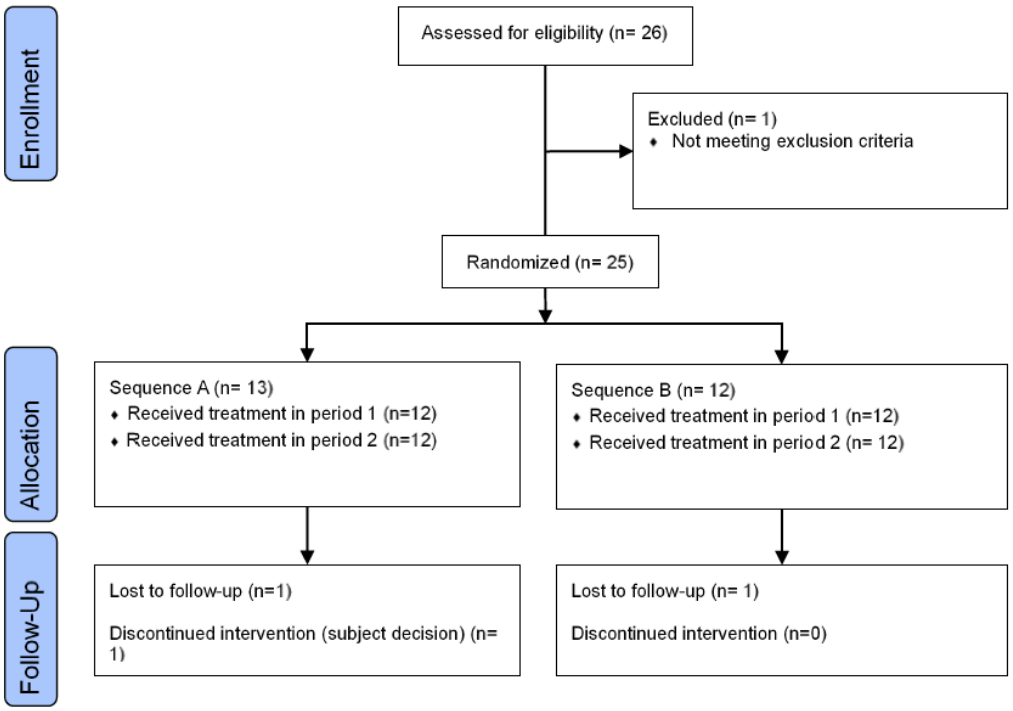
Disposition of Subjects

Twenty-six subjects were screened and 25 subjects enrolled in the study at 4 medical centres in Iran and Turkey between June 2017 and September 2018. Thirteen subjects to sequence A (starting with AryoSeven followed by NovoSeven) and 12 to sequence B (starting with NovoSeven and followed by AryoSeven). Overall, 24 subjects received trial treatment with AryoSeven and NovoSeven and completed the PK-Phase of the study and the following 12 months immunogenicity phase. Last patient out from the immunogenicity phase was in October 2019. Two subjects discontinued trial treatment in the follow-up phase prematurely.

After query resolution, the final decision of study populations was made at the Blinded Data Review Meeting of 23 March 2020, and the blind was broken. The database freeze from 16 March, 2020 was used for the analysis.

The enrolment of subjects with severe FVII Deficiency (FVII<1%) proved very difficult due to the extreme rarity of these subjects. Besides, protocol excluded subjects who have been exposed to AryoSeven before starting study (for Additional group only). The applicant stated that this exclusion criterion made it impossible to recruit subjects for the Additional group in Iran, where all patients with FVII Deficiency are treated with AryoSeven (this study showed that 18/24, 75% of the patients have been treated with AryoSeven in the year before entering the study). In Turkey, also, it was not possible to identify potential candidates by the two participating centres and other three large haemophilia centres in the country. Consequently, the applicant stated that no patients have been enrolled in the Additional group for Immunogenicity. As a result, only the Pharmacokinetic group, that completed the 12 months follow-up, has been analysed, and the results are presented in this report.

Figure 5: Subject disposition



Protocol Deviations

One randomised subject (100201) had 1 major protocol violation which resulted in exclusion from the SES and the PAS population. Due to clotting problems of the sample collected after first treatment administration the investigation was stopped. This subject was re-randomised 3 month later with subject ID 100205 and samples were processed successfully.

Additional protocol deviations considered minor pertained to: time schedule violations, intake of anti-fibrinolytics by three patients, bleeding episodes in the 12 months before entering the study, storage conditions of some sample vials. One patient had a reporting violation pertaining to a SAE not documented or reported during the immunogenicity phase.

Protocol Amendment 1 (dated 15 October 2018) allowed for treatment of bleeding at home. However, all patients started the immunogenicity phase before Protocol Amendment 1 and received AryoSeven for treatment of bleeding on demand at home. In fact, since the beginning of the study, all Investigators decided uniformly to allow for the treatment of bleeding at home. This was, due to the distance of patients' homes from the centre, making it almost impossible for the patients to reach the centre for treatment without increasing the risk of severe complications of bleeding.

Data Sets Analysed

The analysis populations of the protocol included PAS (n=24), SES (n=24) and IAS (n=24) populations.

Pharmacokinetic analysis set (PAS): The PK analysis set (PAS) included subjects who:

- received both the study treatments to which they were randomly assigned, and
- did not have concentrations of FVII ≥1 IU/ml before single dose administration, and
- did not have any major protocol deviations, including a deviation of sampling time at the end of the infusion (i.e. provided an end of infusion sample and at least 12 hours after study drug administration).

Safety evaluation set (SES): The safety evaluation set (SES) included all subjects exposed to at least one dose of the study medication and any documented safety data.

Immunogenicity analysis set (IAS): This set includes all subjects exposed to at least one dose of the study medication and any documented immunogenicity data.

The primary endpoint and secondary endpoints were analyzed for the PAS population.

From the overall 26 screened subjects 25 were randomised and 24 subjects received at least one dose of study drug (=safety population). Analysis sets and reasons for exclusion are summarised in Table 1 below.

Table 1: Summary of subject disposition by sequence

Disposition of patients	Sequence A (N=13)	Sequence B (N=12)	Total N. patients
Subjects Screened	-	-	26
Subjects not randomised	-	-	1
Subjects Randomized	13 (100%)	12 (100%)	25 (100%)
Subjects with protocol deviations	8 (61.5%)	5 (41.7%)	13 (52%)
Subjects with major protocol violations	1 (7.7%)	-	1 (4%)
Subjects completed PK phase	12 (92.3%)	12 (100%)	24 (96.0%)
Subjects discontinued the study [1]	1 (7.7%)	1 (8.3%)	2 (8%)
Safety Population (SES) [2]	12 (92.3%)	12 (100%)	24 (96.0%)
PK Population (PAS)	12 (92.3%)	12 (100%)	24 (96.0%)
Immunogenicity Population (IAS)	12 (92.3%)	12 (100%)	24 (96.0%)

Sequence A: Starting with AryoSeven followed by Novoseven
Sequence B: Starting with Novoseven followed by AryoSeven

(1) Subjects who discontinued the study: 1000201 (Sequence A), 100418 (Sequence B). Disposition of Subjects is reported in Listing 16.2.1

(2) Patient 100201 was excluded from the SES population because he was randomized twice (the second time as 100205) and FVII concentrations data for the first entry are not available

Patient Demographic and Baseline Characteristics

Table 2: Demographic and Baseline Characteristics, by Sequence (SES Population)

	Sequence A (N=12)	Sequence B (N=12)	Total (N=24)
Age			
n	12	12	24
Mean	23.0	27.0	25.0
Std	8.96	9.43	9.23
Median	21.0	24.5	24.0
Q1 - Q3	15.5 - 29.5	21.5 - 30.0	18.5 - 29.5
Min - Max	12 - 41	14 - 45	12 - 45
Age categories			
Pediatric (<18 years)	4 (33.3%)	1 (8.3%)	5 (20%)
≥ 18 years	8 (66.7%)	11 (91.7%)	19 (79.2%)
n	12	12	24
Sex			
Female	6 (50.0%)	7 (58.3%)	13 (54.2%)
Male	6 (50.0%)	5 (41.7%)	11 (45.8%)
n	12	12	24
Race			
White	12 (100%)	12 (100%)	24 (100%)
n	12	12	24
Height [cm]			
n	12	12	24
Mean	167.3	162.2	164.7
Std	9.76	7.52	8.91
Median	168.0	164.0	164.5
Q1 - Q3	162.0 - 173.5	157.5 - 167.0	160.5 - 169.0
Min - Max	146 - 185	146 - 173	146 - 185
Weight [kg]			
n	12	12	24
Mean	71.95	60.45	66.20
Std	20.464	11.387	17.227
Median	65.70	60.10	64.15
Q1 - Q3	59.15 - 79.55	53.38 - 71.93	55.25 - 72.30
Min - Max	45.0 - 116.7	36.0 - 73.0	36.0 - 116.7

N: number of subjects in a sequence group; n: number of subjects; %: percentage based on N; Mean=arithmetic mean; Std=standard deviation; Q1/Q3=1st/3rd quartile; Min=minimum; Max=maximum
Sequence A: Starting with AryoSeven followed by NovoSeven
Sequence B: Starting with NovoSeven followed by AryoSeven

Overall, the sequence groups were well balanced concerning the demographic data at baseline. Results for the PAS population were very similar.

Demographic and baseline of the safety population were not considered different from that of the PK analysis population

History of Bleeding

The history of bleeding episodes and treatment information baseline disease characteristics were also similar between the sequence groups. The mean number of bleeding events during the last 12 months was 22.8 ± 39.5. In 21 (87.5%) subjects the cause for the last/most recent bleeding episode was spontaneous and in 3 subjects the cause was traumatic. In 18 subjects this last/most recent bleeding episode was treated with AryoSeven.

Medical History

On a MedDRA SOC basis (using MedDRA V21.0), the most frequent previous diseases (i.e. diseases not ongoing at the start of study treatment) were surgical and medical procedures. The most frequent concomitant diseases were congenital, familial and genetic disorders (3 of 24 subjects reported at least one event).

Pharmacokinetics Analysis

A total of 24 subjects were included in the analysis. All 24 subjects completed both periods.

Primary Pharmacokinetics Variables

The primary objective was to demonstrate bioequivalence between AryoSeven and NovoSeven based on the primary pharmacokinetic endpoint area under the plasma activity-time curve from time 0 to last quantifiable activity AUC_{last} and maximum plasma activity C_{max}. The primary endpoints were based on factor VIIa (FVIIa) activity measurements. To demonstrate bioequivalence between the two formulations (AryoSeven and NovoSeven), a linear model was applied to the logarithmically transformed (AUC_{last}) and maximum plasma activity (C_{max}). The model included treatment, period and sequence as fixed effects and subject as a random effect. The ratios of AryoSeven/NovoSeven with two- sided 90% confidence interval were estimated using least square means. If the 90% CI were completely contained within the equivalence interval (0.8-1.25), the two treatments were to be considered bioequivalent.

The results were confirmed by two one-sided t-tests on the 5% α-level. To exclude that there was a significant period or sequence effect an ANOVA was performed to evaluate these effects.

The primary endpoints AUC_{last} and C_{max} were based on the FVIIa activity. Predosing, FVIIa activity ranged from <0.00381 to 0.00486 IU/mL for AryoSeven and from <0.00381 to 0.05636 IU/mL for NovoSeven. Ten minutes after dosing with AryoSeven maximal activity was observed in all subjects. After dosing with NovoSeven, in 23 subjects maximal activity was observed after 10 minutes, subject 100402 reached C_{max} after 20 minutes. Decrease in FVIIa activity was similar between formulations. The Statistical Analysis Bioequivalence assessment is reported in Table 3 below.

Table 3: Summary Statistics for Primary PK Endpoints, AUC_{last} and C_{max}

Treatment	AUC_{last} (h x IU/mL)	C_{max} (IU/mL)
AryoSeven (n=24)		
Mean	20.29	11.49
Std	6.19	2.17
Geometric mean	19.57	11.29
95% CI of geometric mean	17.17 – 22.04	10.41 -12.25
% CV	30.5	18.9
NovoSeven (n=24)		
Mean	20.65	11.51
Std	7.12	2.82
Geometric mean	19.56	11.15
95% CI of geometric mean	16.96 – 22.56	9.99 – 12.46
% CV	34.5	24.5

Mean=arithmetic mean, Std=standard deviation, CV=coefficient of variation, 95% CI=95 % Confidence Interval - Source: Table 14-2-1-2

For AUC_{last} [h*IU/mL], 95% CI 0.9313, 1.0619 with ANOVA and 95% CI 0.9205, 1.0744 with two one-sided t-tests. All the CI are completely contained within the bioequivalence interval (0.80–1.25) and the two formulations are considered bioequivalent. For C_{max} [IU/mL], 95% CI 0.951, 1.0779 with ANOVA and 95% CI 0.9404, 1.09 with two one-sided t-tests. All the CI are completely contained within the bioequivalence interval (0.80–1.25) and the two formulations are considered bioequivalent. Table 3

shows that geometric means for AUC_{last} were equal for both formulations. The geometric mean for C_{max} was slightly higher for AryoSeven. For both parameters the variability (%CV) was about 5% higher for NovoSeven.

The mean AryoSeven/NovoSeven ratio of AUC_{last} was 0.9945, 90% CI (0.9313 – 1.0619), within the predefined equivalence range. The mean AryoSeven/NovoSeven ratio of C_{max} was 1.0124, 90% CI (0.951 – 1.0779), within the predefined equivalence range. These results from the ANOVA approach were confirmed by the two one-sided t-tests.

Secondary Pharmacokinetics Variables

Secondary pharmacokinetic parameters are summarised in Table 4. AUC and C_{max} were also evaluated dose normalised as secondary pharmacokinetic parameters ($AUC_{last\text{-}norm}$, $AUC_{inf\text{-}norm}$ and $C_{max\text{-}norm}$).

All secondary parameters (AUC_{inf}, $AUC_{inf\text{-}norm}$, AUC_{extra}, t_{max}, $t_{1/2}$, MRT, CL_{sys}, V_d , including $AUC_{last\text{-}norm}$ and $C_{max\text{-}norm}$) were comparable between the two formulations. The mean AryoSeven/NovoSeven ratios with 90% CI are listed in Table 5 below.

The 90% CI were within the equivalence range (0.8-1.25) for all parameters with the exception of AUC_{extra} (90% CI 0.8122 1.6573).

Table 4: Summary Statistics for Secondary Pharmacokinetic Endpoints

Treatment	AUC_{inf} (h x IU/mL)	t_{max} (h)	$t_{1/2}$ (h)	MRT (h)	CL_{sys} (mL/h)	V_d (mL)
AryoSeven (n=24)						
Mean	20.39	0.18	1.71	1.91	104.5	246.2
Std	6.22	0.005	0.51	0.39	35.2	73.4
Geometric mean	19.54	0.17	1.65	1.88	98.6	235.3
% CV	30.5	2.9	29.7	20.2	33.7	29.8
NovoSeven (n=24)						
Mean	20.74	0.18	1.66	1.94	103.6	240.3
Std	7.12	0.033	0.46	0.41	35.6	90.0
Geometric mean	19.65	0.18	1.61	1.91	98.0	228.1
% CV	34.3	18.0	27.9	21.0	34.4	37.5

Mean=arithmetic mean; Std=standard deviation; CV=coefficient of variation
Source: Table 14-2-1-2

Table 5: Secondary Pharmacokinetic Analysis: Point estimates and 90% Confidence Intervals

Parameter	Statistical Method	Point estimate	Confidence limits		Level of confidence
			lower	upper	
AUCinf [h*IU/mL]	ANOVA	0.9941	0.9313	1.0611	0.9
AUCinf-norm [h*IU/mL]		0.9929	0.9302	1.06	
AUClast-norm [h*IU/mL]		0.9962	0.933	1.0637	
AUCextra [%]		1.1602	0.8122	1.6573	
CLsys [mL/h]		1.0059	0.9424	1.0737	
Cmax [IU/mL]		1.0124	0.951	1.0779	
Cmax-norm [IU/mL]		1.0106	0.9494	1.0757	
Lz [h^-1]		0.9751	0.9289	1.0236	
t1/2 [h]		1.053	0.9543	1.1619	
MRT [h]		0.9692	0.9189	1.0223	
Vd [mL]		1.0316	0.9497	1.1205	
tmax [h]	Wilcoxon signed rank test	0	0	0	

3.3.1.2. Pharmacodynamics

Mechanism of action

The mechanism of action includes the binding of factor VIIa to exposed tissue factor, in the presence of both calcium and phospholipids. This complex activates factor X into factor Xa, bypassing the reactions that require factor VIII (FVIII) or factor IX (FIX) and leading to the initial conversion of small amounts of prothrombin into thrombin. Thrombin leads to the activation of platelets and factors V and VIII at the site of injury and to the formation of the haemostatic plug by converting fibrinogen into fibrin. Pharmacological doses of HemAryo activate factor X directly on the surface of activated platelets, localised to the site of injury, independently of tissue factor. This results in the conversion of prothrombin into large amounts of thrombin independently of tissue factor.

Primary and Secondary pharmacology

Pharmacodynamic was a primary endpoint in Study **UGA 2014-01**.

PD endpoints

The following PD parameters were measured on all patients 10 min- prior to dose administration (baseline), and 10 min, 20 min, 1 h, 3 h, 5 h, 8 h and 12 h, 24 h and 30 h after drug administration for TGA testing and 20 min, 1 h, 5 h, 12 h and 24 h after injection for D-dimer and F1.2:

Thrombin Generation Assay (TGA):

- AUClast of Peak height (nmol/L)
- AUClast of Endogenous thrombin potential (ETP): calculated as area under the curve TGA (nmol/L per min) from baseline to last measured value post-dosing
- AUClast of Lag time (time to 16.7% of peak concentration in minutes)

According to the applicant, additional parameter of TGA time to peak, time to tail, and KIIa reaction rate have been also assessed but were not part of the efficacy analysis.

D-dimer:

- Peak height (µg/L)
- AUCDdim: area under the curve (µg/L per min) calculated from baseline to last measured value post-dosing

Prothrombin fragments F1.2:

- Peak height (ng/L)
- AUCF1.2: area under the curve (ng/L per min) calculated from baseline to last measured value post-dosing

Laboratory personnel of the central lab was blinded to the treatment received by the patient.

Results

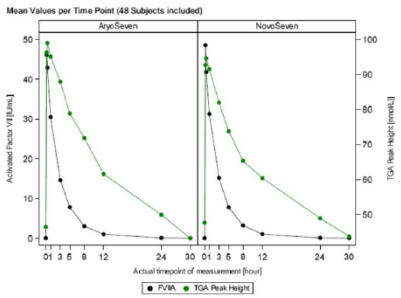
PK/PD relationship between either TGA, D-dimer, F1.2 and FVII:C concentrations have been explored graphically only.

As seen in Figure 11-5, TGA peak height and ETP showed a similar increase with a peak similar to Cmax of the FVII clotting activity. Levels returned to baseline around 30 hours post infusion. The lag-time in thrombin formation also shows a similar curve between the two drugs. D-dimer levels show a similar trend between the drugs until the 12 hours measurement time-point. Prothrombin fragments 1+2 (F1+2) showed an increase with a peak similar to Cmax, indicating the formation of thrombin in the patient after administration of rhFVIIa.

Figure 6

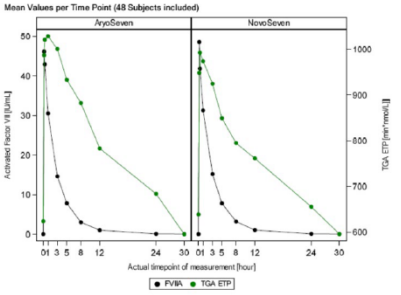
Figure 11-5. Mean Relationship between TGA Lag Time, ETP, Lag Time, D-dimer, F1-2 and FVII clotting activity after Administration of AryoSeven or NovoSeven (Linear Scale)

TGA Peak Height



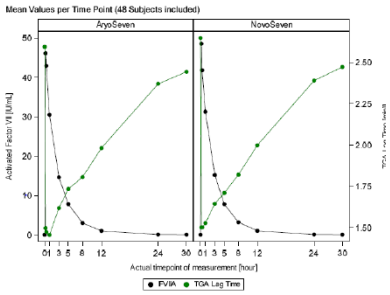
Source: Figure : Figure 14.13.3.1

TGA ETP



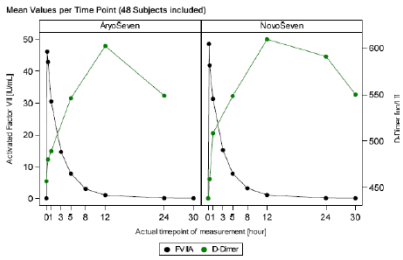
Source: Figure 14.14.3.1

TGA Lag Time



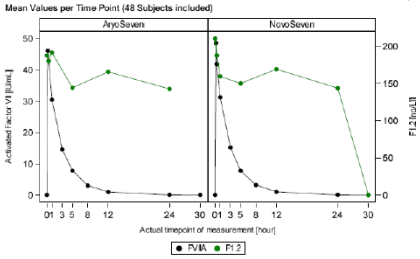
Source: Figure 14.15.3.1

D-dimer



Source: Figure 14.16.3.1

F1.2



Source: Figure 14.17.3.1

Primary PD endpoints

- **Thrombin Generation**

Table 11-8. Bioequivalence assessment of primary PD endpoints Thrombin Generation Assay

Parameter	Dose level	Statistical Method	Point estimate	Confidence limits		PD_{marg}		CV
				lower	upper	lower	upper	
TGA ETP (AUC)	90	ANOVA	1.1444	1.0629	1.2322	0.8	1.25	0.1006
	270	ANOVA	1.038	0.9888	1.0997	0.8	1.25	0.1027
TGA Lag Time (AUC)	90	ANOVA	1.0704	0.9492	1.2071	0.8	1.25	0.2395
	270	ANOVA	0.9672	0.924	1.0124	0.8	1.25	0.092
TGA Peak height (AUC)	90	ANOVA	1.1279	1.0543	1.2066	0.8	1.25	0.0976
	270	ANOVA	1.0446	1.0048	1.086	0.8	1.25	0.076

For the primary PD endpoints TGA ETP (AUC), TGA Lag Time (AUC) and TGA Peak height (AUC), the 90% CI of the ratios of AryoSeven/NovoSeven of both doses tested (90 µg/kg and 270 µg/kg) are completely contained within the bioequivalence interval of (0.8–1.25). However, the resulting 90% CIs for TGA ETP and TGA peak heights not covering "1" indicates a statistically significant difference in these parameters.

- **D-dimer**

Table 11-9. Bioequivalence assessment of primary D-dimer endpoints (PK/PD Analysis Set)

Parameter	Dose level	Statistical Method	Point estimate	Confidence limits		PD_{marg}		Coefficient of variation
				lower	upper	lower	upper	
$AUC_{D\text{-}dimer}$	90	ANOVA	0.9813	0.7371	1.3064	0.8	1.25	0.1625
	270	ANOVA	0.8416	0.633	1.1189	0.8	1.25	0.1392
Peak D-dimer	90	ANOVA	1.0077	0.9645	1.0527	0.8	1.25	0.1085
	270	ANOVA	0.9895	0.9151	1.0699	0.8	1.25	0.1679

The results of the AUC comparison showed that the 90% CIs were outside of the proposed lower bound of the equivalence margin of (0.80 to 1.25) for both concentrations.

Peak value comparisons resulted in a 90% CI within the 80% to 125% limit.

- **F1.2 prothrombin fragments**

Table 11-10. Bioequivalence assessment of primary PD endpoints F1.2 (PK/PD Analysis Set)

Parameter	Dose level	Statistical Method	Point estimate	Confidence limits		PD_{marg} *		Coefficient of variation
				lower	upper	lower	upper	
$AUC_{F1.2}$	90	ANOVA	1.0364	0.7864	1.3659	0.7063	1.4159	0.4675
	270	ANOVA	1.0657	0.6937	1.6372	0.8	1.25	0.2467
Peak F1.2	90	ANOVA	1.1668	1.0202	1.3343	0.6524	1.5327	0.5741
	270	ANOVA	0.92	0.7665	1.1043	0.7797	1.2826	0.3346

Source: Table 14.2.3.4.3; * for PD_{marg} definition, please refer to section 11.4.1.2.

The applicant has widened the equivalence margins for the peak value of F1.2 and for the AUC of the lower dose without adequate justification (see Statistical methods in Section 3.3.1.1.), therefore this parameter is not assessed. For the higher dose of 270 µg/kg, the applicant used the standard margin of 0.8 to 1.25. The point estimate for AUC was 1.0657 (90% CI: 0.6937 to 1.6372), where the 90% CI were outside of the proposed lower bound of the margin. Therefore, HemAryo and NovoSeven are not comparable for the PD endpoint F1.2.

High variability emerged from the entire (co-primary) PD parameter set chosen by the applicant, and similar results could only be obtained for TGA lag time and D-dimer peak. Furthermore, no results were presented for the additional (secondary) endpoints. For a robust interpretation in an equivalence setting, the results of the PD comparisons should lead to similar conclusions, taking into account an adequate equivalence margin chosen for statistical and clinical reasons, which was not provided. Based on the grounds presented, the study has formally failed to show equivalence in PD between HemAryo/AryoSeven and NovoSeven. The applicant should discuss why a lack of PD comparability should not be considered clinically relevant with regard to the chosen endpoints and the different doses used.

3.3.2. Discussion on clinical pharmacology

Design and conduct of clinical studies

The applicant has submitted two studies that included the assessment of pharmacokinetics to support a comparative evaluation between HemAryo/AryoSeven and NovoSeven. The pivotal Phase III study

UGA 2014-01 was conducted in 48 male adolescent and adult patients with congenital haemophilia A or B with inhibitors applying two doses (90 µg/kg and 270 µg/kg). The **UGA 2014-02** study was conducted in 24 adolescent and adult, male patients with congenital FVII deficiency.

Both were multicentre, randomised, double-blinded, single-dose, cross-over clinical trials comparing AryoSeven and the originator NovoSeven from the EU market. The cross-over phases were designed to evaluate PK/PD comparability and study **UGA 2014-02** was designed to include an additional group for immunogenicity assessment. At the end of the PK/PD phase, all patients entered a single-arm open phase up to 12 months to assess efficacy, safety and immunogenicity of the biosimilar candidate HemAryo.

In both **UGA 2014-01** and **UGA 2014-02**, subjects were recruited and randomly assigned to receive either a single dose of AryoSeven followed by a single dose of NovoSeven or vice versa. Two doses were tested in **UGA 2014-01** (90 µg/kg and 270 µg/kg in a two-way cross-over design), while a dose of 30 µg/kg was tested in **UGA 2014-02**. The dosing was agreed with the EMA CHMP and corresponds to the currently approved posology for the representative patient population.

The design of the PK/PD phase with a washout period of 3 days is appropriate to avoid any carry-over effect as this period covers more than 30 elimination half-lives (t½=2.3 h) in haemophilia patients and more than 24 elimination half-lives in FVII deficiency patients (t½ = 3 h). Patients in routine prophylaxis with Novoseven or AryoSeven (in Iran) were requested to stop prophylactic treatment 3 days before receiving the first dose of study medication; this is acceptable for the PK comparison, since the 3 days is considered a sufficiently long washout period.

The test and reference products differed in vial size and strength, i.e. 1.2 mg (0.6 mg/ml) and 1.0 mg (1.0 mg/ml), respectively. In view of the fact that the original NovoSeven formulation is apparently no longer available, it was previously agreed that AryoSeven 1.2 mg may be compared with the NovoSeven 1mg "room temperature stable" formulation provided it can be shown by quality data that the two NovoSeven formulations and AryoSeven are sufficiently similar (Please see section 3.1.3.5. for details). Due to the differences in the formulation and to the limitation in repackaging of approved products, blinding was performed by an independent third-party operator (nurse/pharmacist, unblinded) who prepared indistinguishable syringes with patients' dosing and labelling. A specific procedure was prepared and distributed to all centres, providing an adequate training to the centre's personnel.

Efficacy, safety and immunogenicity aspects were planned to be assessed during the 12-month follow up phase. The applicant was previously advised to include only patients minimally exposed to AryoSeven that is 1-5 previous exposure days, in order to be able to conduct a proper immunogenicity evaluation. However, this was not considered in the inclusion/exclusion criteria for the PK groups of the studies. Furthermore, the exclusion of pre-existing antibodies against FVII poses challenges regarding the immunogenicity assessment. Therefore, immunogenicity data obtained in the PK group are of limited value.

A separate group of patients previously untreated with AryoSeven was planned for the immunogenicity evaluation of **UGA 2014-02**. Previously, it was agreed with EMA that the monitoring of immunogenicity and other safety parameters would start pre-authorisation and be completed post-marketing (EMA/CHMP/SAWP/168901/2015). However, this part of the study was not carried out, which constitutes a major protocol deviation (see section 3.3.7 Clinical safety for more information).

Generally, the study designs are considered appropriate to sensitively detect potential PK differences between HemAryo/AryoSeven and the reference product NovoSeven.

Study UGA 2014-01

Male adult and paediatric patients (>12 years) with haemophilia A or B with inhibitors titer >5 Bethesda Units [BU] and with > 2 episodes of bleeding requiring treatment with FVII infusions within the previous 12 months at trial start, not in bleeding status were included.

The exclusion of patients with antibodies against factor VII is advantageous for the PK assessment, but generally rather disadvantageous for the assessment of immunogenicity. Therefore, the immunogenicity data obtained in UGA 2014-01 are of limited value.

Parallel participation in another experimental drug trial or another marketed drug trial that may affect the primary study endpoint were excluded, however the applicant should reassure that no experimental medicinal products that could potentially harm the study have been taken prior to the start of the study.

The study was completed in October 2020, but specific dates are missing. The exact date of the end of the study should be announced. The majority of patients come from non-EEA countries and the largest enrolling centre was in Iran. There is evidence that haemophilia A and B occurs without ethnic predominance and there does not appear to be major ethnic variations of mutations in haemophilia A and B. Nevertheless, the applicant should provide a thorough discussion that there are no significant ethnic variations. Regarding differences in practice, the applicant gave an overview of clinical practice in Iran versus Europe. There is considerable overlap in clinical practice, especially with regard to the recent introduction of prophylaxis protocols in Iran. Standard treatment regimens seem comparable.

Two protocol amendments were indicated, most of them were included following regulatory advice by EMA. As the first amendment (dated 5 October 2018) was introduced after the enrolment of the first patient (17 September 2018), the applicant should explain whether the treatment phase of the patients has already started and how many/which patients were affected. The applicant should also explain whether the amendment for Turkey (dated 5 March 2019) includes only the national amendment or the overall amendment (protocol amendment #1). According to the data presented, most of the protocol violations were due to the COVID19 pandemic and affected the assessment of secondary endpoints in the follow up phase. This resulted in an increased number of delayed control visits to the Investigator. The classification as "minor" deviations can be considered acceptable, as it does not affect the evaluation of the primary endpoint. However, it should be explained what is meant by "washout period of 3 days (before protocol amendment 1)" in the case of patient 100401. It was acknowledged in a refusal that in various sections reference was made to two primary PK parameters AUCinf and Cmax, incorrectly stating "maximum plasma activity Cmax" as the primary endpoint. The applicant should therefore justify if Cmax was used as the primary endpoint at the start of the study.

PK comparability

In both pivotal comparative studies, PK assessment was made by measurement of plasma level of circulating factor VII using a clotting method, the commercial Staclot VIIa–recombinant tissue factor assay. Overall, the most important validation parameters were sufficiently addressed in this partial validation, but some key details were not included in the validation report.

The planned sampling times were before dosing and at 10 min, 20 min, 1 h, 3 h, 5 h, 8 h, 12 h, 24 h and 30 h post-dose. The mean terminal half-life of eptacog alfa was found to be 2.3 hours (SmPC NovoSeven), therefore sampling time points are supported.

In **UGA 2014-01**, AUC_{inf} was selected as the primary PK parameter that is consistent with EMA guidance EMEA/CHMP/BMWP/42832/2005 Rev1 and is acceptable. Secondary PK endpoints including AUC_{last}, C_{max}, CL, t_{max}, $t_{1/2}$, V_{ss} and V_z are supported as well. In **UGA 2014-02** AUC_{last} and C_{max} were

selected as primary PK parameters. In principle, it is recommended to evaluate $AUC_{0\text{-}inf}$ as primary PK parameter in a single dose PK study with intravenous administration. However, the selection of AUC_{last} and C_{max} as the primary PK parameters is acceptable, since $AUC_{0\text{-}inf}$ was assessed as secondary PK endpoint. Thus, overall the choice of the primary and secondary PK parameters is considered adequate to demonstrate similarity between HemAryo and the reference product.

The applicant has assessed the bioequivalence in the primary PK parameters between Aryoseven and Novoseven on the basis of the 90% confidence interval for the ratio of the population geometric means (test/reference) for the parameters AUC_{last} and C_{max}. The PK parameters were analysed using ANOVA. Bioequivalence was considered to be established if the 90% confidence interval was entirely within the margin of 0.8 and 1.25.

The chosen method of analysis is considered appropriate and the chosen margins are in accordance with the respective guidelines. The sample size is reproducible given the underlying assumptions. AUC_{extra} and the fraction of AUC_{inf} that is given by AUC_{last} is considered a crucial secondary parameter. No concerns arise form a methodological point of view.

Results

Study UGA 2014-01

From the overall 53 screened subjects, 49 were randomised and 48 subjects received at least one dose of study drug. The study population consisted of 48 patients with haemophilia A/B with inhibitors. All 48 subjects completed the PK/PD cross-over phase and were included in the PK/PD analysis set (PAS).

The distribution of demographic and baseline characteristics is not well balanced in age, BMI (height and weight) or bleeding rates. The applicant is asked to provide more details on the randomisation process and discuss a potential impact of differences in age, weight and bleeding between the study groups on the results of the study.

For AryoSeven and the reference product NovoSeven, one patient who received a dose of 90 µg/kg (subject ID: 200401; sequence A90) had particularly high concentration values at the time point 10 minutes after administration compared to all other patients, where e.g., concentration values ranged from 50 to 100 IU/ml. In contrast, the C_{max} achieved in this patient were about 150 IU/ml and 200 IU/ml after treatment with AryoSeven and NovoSeven, respectively. The applicant should investigate and explain the reasons for the increase in C_{max} after administration of the IMPs HemAryo and NovoSeven. If a data error has occurred, the applicant should recalculate the data analyses for the PK comparison.

Similarity between AryoSeven and NovoSeven could be demonstrated, as the 90% CI for the geometric LS means ratios of AUC_{inf} were fully contained within the predefined bioequivalence limits of 0.80 to 1.25. The point estimates for AUC_{inf} for the 90 µg/kg was 0.9995 with a 90% CI of (0.9805, 1.019) and for 270 µg/kg the point estimate was 0.9631 with a 90% CI of (0.917, 1.0116). In addition, the secondary endpoints were largely in support of PK similarity.

Study UGA 2014-02

From the overall 26 screened subjects, 25 were randomised and 24 subjects received at least one dose of study drug. The study population consisted of 24 patients with FVII deficiency. All 24 subjects completed the PK cross-over phase and were included in the PK analysis set.

The distribution of demographic and baseline characteristics is not well balanced in age, BMI (height and weight) or bleeding rates. The applicant is asked to provide more details on the randomisation process and discuss a potential impact of differences in age, weight and bleeding between the study groups on the results of the study.

According to the list of protocol violations, one SAE that happened during the immunogenicity phase was not documented or reported. The applicant should specify what kind of event occurred and provide all available details, if not already presented in the safety part of the study report.

HemAryo and the reference product NovoSeven were considered bioequivalent in terms of the primary endpoints C_{max} and AUC_{last} as the 90% CI for the geometric LS means ratios were fully contained within the predefined bioequivalence limits of 0.80 to 1.25. It is, however, unclear if the 90 or 95% CI was used for the PK comparison, since different data are given in the clinical study report. The applicant is asked to clarify this.

Although it is generally recommended to evaluate $AUC_{0\text{-}inf}$ as primary PK parameter in a single dose PK study with intravenous administration, AUC_{last} and C_{max} are also considered sensitive parameters to investigate PK biosimilarity for eptacog alfa. Furthermore the geometric LS means ratio was also provided for $AUC_{0\text{-}inf}$. As the observed results fall into the predefined equivalence margins of 80-125% for the 90% CI of each of the parameters, the results of this study are suggestive of PK similarity of HemAryo and NovoSeven. The applicant collected a series of supportive PK data in this pivotal PK trial. Even though not required for secondary PK parameters the applicant presented the mean AryoSeven/NovoSeven ratios with 90% CI for all of the secondary parameters. The 90% CIs were within the equivalence margin for all secondary PK parameters (AUC_{inf}, $AUC_{inf\text{-}norm}$, t_{max}, $t_{1/2}$, MRT, CL_{sys}, V_d, $AUC_{last\text{-}norm}$ and $C_{max\text{-}norm}$), except for AUC_{extra}. The 90% CI for AUC_{extra} was 0.8122–1.6573 and therefore not entirely within the 90% CI of 0.80–1.25. However, this might not be clinically relevant, since more than 80% of the AUC is covered by the PK sampling time points.

PD comparability

The applicant has conducted the PD assessment in study **UGA 2014-01**. During the EMA-SA, it was recommended to employ extended comparative PD characterisation in the proposed Phase III clinical trial in patients with haemophilia and inhibitors of VIII/IX. This was considered important as this part of the biosimilarity exercise will substitute relevant clinical comparative data and the extended comparative PK/PD is therefore crucial for the demonstration of biosimilarity. The decision not to investigate PD in patients with congenital FVII deficiency was accepted as the PD evaluation in UGA 2014-01 was considered sufficient. However, it was noted in the EMA-SA that an assessment of PD parameters in the factor FVII deficiency patient population would support the biosimilarity claim (if similar results were obtained).

The applicant has analysed the PD activities of HemAryo across the two different dose levels of 90 µg/kg and 270 µg/kg. The used PD endpoints (TGA, D-dimer and F1.2 prothrombin fragments) are not accepted surrogate markers for efficacy, however it is acknowledged that they may be relevant for the pharmacological action of the active substance. No justification was provided as to why these endpoints in particular were selected.

The applicant performed a partial validation of commercially available assays to assess thrombin generation and D-dimer content. Further information about the matrix in which the validation was performed is required to confirm the applicability of both assays. Regarding applied prothrombin fragment 1+2 content assay, is unclear if performed partial validation sufficiently assures the applicability of the assay, since again no information about applied validation matrix, and validation activities performed by the commercial supplier of the assay was available.

PK/PD relationship between either TGA, D-dimer, F1.2 and FVII:C concentrations have been explored, but analysis of PK/PD relationship is currently vague, since the data have been explored only graphically and it is not clear if such relationship could be used to set the acceptance margins.

The applicant has not provided any justification for the chosen equivalence margins. In addition, the usual PK margins of 0.8 and 1.25 respectively were inappropriately widened. Finally, the applicant has

used the 90% confidence intervals instead of 95% for the primary PD analysis, which is not considered acceptable.

Against the background of the major methodological criticism expressed in relation to:

(i) the coverage probability of confidence intervals used for PD similarity assessment (90% used instead of desired 95%) and

(ii) the inadequacy of the choice of equivalence margins to evaluate PD equivalence (no clinical justification for using 0.8-1-25, and unacceptable and flawed approach for widening)

the applicant is asked to comment in which way those methodological concerns can be overcome to allow for a sound similarity evaluation of the predefined primary PD parameters.

Results

For the primary PD endpoints TGA ETP (AUC), TGA Lag Time (AUC) and TGA Peak height (AUC), the 90% CI of the ratios of AryoSeven/NovoSeven of both doses tested (90 µg/kg and 270 µg/kg) are contained within the bioequivalence interval of (0.8–1.25). However, the resulting 90% CIs for TGA ETP and TGA peak heights not covering "1" indicates a statistically significant difference in these parameters. Additional parameter of TGA time to peak, time to tail, and KIIa reaction rate have been also assessed but were not part of the analysis. The applicant is therefore asked why these results were not included.

The peak value comparisons for D-Dimer resulted in a 90% CI within the 80% to 125% limit, whereas the results of the D-Dimer AUC comparison demonstrated that the 90% CIs were outside of the proposed lower bound of the equivalence margin of (0.80 to 1.25) for both concentrations. The applicant has widened the equivalence margins for the peak value of F1.2 and for the AUC of the lower dose without adequate justification, therefore this parameter was not assessed. For the higher dose of 270 µg/kg, the standard margin of 0.8 to 1.25 was used, however the 90% CI were outside of the proposed lower bound of the margin. High variability emerged from the co-primary PD parameter set, and similar results could only be obtained for TGA lag time and D-dimer peak. For a robust interpretation in an equivalence setting, the results of the PD comparisons should lead to similar conclusions, taking into account an adequate equivalence margin chosen for statistical and clinical reasons, which was not provided. Based on the grounds presented, the study has formally failed to show equivalence in PD between HemAryo/AryoSeven and NovoSeven. The applicant is asked a) to comment in which way those methodological concerns can be overcome to allow for a sound similarity evaluation of the predefined primary PD parameters and b) to discuss why a lack of PD comparability should not be considered clinically relevant with regard to the chosen endpoints and the different doses used.

3.3.3. Conclusions on clinical pharmacology

The higher plasma concentrations observed in one patient after administration of both IMPs compared to all other patients in study UGA 2014-01 require further justification. If this concern can be eliminated, PK similarity can be concluded based on the results of the pivotal studies UGA 2014-01 and UGA 2014-02.

Similarity in PD between HemAryo and EU-NovoSeven cannot be concluded as the co-primary endpoints did not meet the predefined equivalence criteria. Further analyses and a discussion on the clinical relevance of the observed results is needed before a final conclusion on similarity on PD level can be drawn.

3.3.4. Clinical efficacy

3.3.4.1. Dose-response studies

Not applicable for biosimilars.

3.3.4.2. Main study(ies)

Clinical efficacy data are available from 2 pivotal Phase III studies:

- The pivotal trial **UGA 2014-01** was a randomised, multicentre, double blind clinical trial comparing PK, PD and safety of HemAryo and NovoSeven in 48 patients with haemophilia A or B with inhibitors (detailed description of the study design and conduct is presented in section 3.3.1.1 of this report).
- Pivotal trial **UGA 2014-02** was a randomised, multicentre, single dose, cross-over, double blind study comparing the pharmacokinetic of HemAryo with NovoSeven in 24 patients with congenital factor VII deficiency (see also section 3.3.1.1).

Clinical response in the treatment of bleeding during the follow-up immunogenicity phase was a secondary efficacy parameter.

As only a weak exposure-response relationship has been described for eptacog alfa and efficacy studies are unlikely to be sensitive enough to detect differences, the company has considered extended comparative PK/ PD characterisation for the demonstration of biosimilarity. Information on efficacy within these trials is considered supporting information. As already discussed during the EMA-SA (EMA/CHMP/SAWP/168901/2015), the usual considerations for an equivalence trial should be observed. The applicant mainly collected efficacy data in the immunogenicity follow-up period without a comparator.

Efficacy Endpoints

Efficacy was assessed as a secondary endpoint in the Phase III studies **UGA 2014-01** and **UGA 2014-02** in 48 haemophilia patients and 24 FVII deficient subjects, respectively.

At the end of the PK/PD phase (visit 3), patients received a number of vials of AryoSeven to treat bleeding or as prophylaxis for the following 12 months. Treatment with AryoSeven during this follow-up phase was administered at home, with dose and duration of treatment based on the Investigator's decision, and iv. self-injection either by parents, caregiver or a support person.

All subjects were trained by the centres' personnel on how to use the 4 points scale for assessing the response to the treatment with AryoSeven at home, how to record the results on a dedicated form, and were requested to bring the form to every 3-monthly visit for discussion with the Investigator. The Investigators reviewed these forms at every control visit, and discussed them with the patients before confirming.

Study UGA 2014-01: For every bleeding episode that occurred during the 12 months, patients received AryoSeven 90 µg/kg for one or more doses until resolution of bleedings (dose and duration of treatment was based on the Investigator's decision).

Study UGA 2014-02: Patients received treatment with 30 µg/kg AryoSeven (for one or more days until resolution of bleeding) for every bleeding episode that should occur during 12 months – or – for prophylaxis with AryoSeven, with dose and frequency that should be modified based on Investigator's decision.

Efficacy was evaluated for each treatment modality at 2, 6 and 12 hours post-infusion (last dose of AryoSeven) using a 4 point scale rating for assessment of treatment response (Excellent, Good, Moderate, None). Treatment was assessed as failure if at least 3 doses of AryoSeven were administered. Prophylaxis with AryoSeven was allowed, with dose, frequency of administration and duration based on the Investigator's decision.

Four points scale for assessment of treatment response was as followed:

Excellent: Full relief of pain and cessation of objective signs of bleeding (i.e. swelling, tenderness, and decreased range of motion in the case of musculoskeletal haemorrhage). No additional infusion was required for the control of bleeding. Administration of further infusions to maintain haemostasis would not affect this scoring.

Good: Definitive pain relief and/or improvement in signs of bleeding after the third infusion.

Moderate: Probable and/or slight relief of pain and slight improvement in signs of bleeding after the third infusion.

None: No improvement or condition worsened.

The treatment response at both time points has been evaluated in original scale and as binary response (Responder: Excellent/Good and Non-responder: Moderate/None).

Number of bleeding episode requiring treatment with AryoSeven, number of days of exposure, number of doses administered to stop bleeding were planned to be analysed with descriptive statistics.

In case of absence of response, plasma samples were taken to investigate the presence of antibodies against FVII.

Results

Study UGA 2014-01

Efficacy was assessed for all subjects in the IAS.

Bleeding history: The mean (±SD) number of bleeding events during the last 12 months was 22.3 ± 24.97 (min-max: 3-100 bleeding episodes). In 31 (64.6%) subjects the cause for the last/most recent bleeding episode was spontaneous and in 17 (35.4%) subjects the cause was traumatic. In 18 patients (37.5%) the last/most recent bleeding episode was treated with HemAryo, and 19 patients (39.6%) received FEIBA.

Data were available for 42 patients as evaluation of 6 patients is still ongoing at different duration of their follow-up (subject ID: 100467, 100468, 100469, 100605, 100606, 100607). All 42 patients had bleeding during the Immunogenicity Phase.

One bleeding event was reported during the PK/PD phase. During the immunogenicity phase there were 2236 single administrations of AryoSeven for treatment on demand of 863 bleedings.

Most bleedings were treated at home, but four patients (subject ID: 100414, 100416, 200301, and 200401) had to be hospitalised for six bleedings that were all resolved. Twenty-three patients received AryoSeven for prophylaxis of bleeding.

Table 8. Treatment Response of Bleeding Episodes during the 12 months follow-up (UGA 2014-01)

	Bleeding response (N=42*)
Number of bleeding episodes per patient:	
Number of patients	42
Mean	20.6
Std	18.64
Median	17
Min-Max	1-100
Total number of bleedings	863
Treatment response 2 h post infusion:	
Non-responders	113 (13.1%)
• None#	4 (0.5%)
• Moderate#	109 (12.6%)
Responders	750 (86.9%)
• Good#	532 (61.6%)
• Excellent#	218 (25.3%)
n	863
Treatment response 6 h post infusion:	
Non-responder	51 (5.9%)
• None#	1 (0.1%)
• Moderate#	50 (5.8%)
Responder	812 (94.1%)
• Good#	159 (18.4%)
• Excellent#	653 (75.7%)
n	863
Treatment response 12 h post infusion:	
Non-responder	34 (3.9%)
• None#	1 (0.1%)
• Moderate#	33 (3.8%)
Responder	829 (96.1%)
• Good#	32 (3.7%)
• Excellent#	797 (92.4%)
n	863

863 bleedings were treated with 2236 single administrations of AryoSeven, with a mean of 20.6 bleedings per patient. Almost all bleedings were completely resolved within 12 hours (829/863, 96%).

Study UGA 2014-02

Efficacy was assessed for all subjects in the IAS.

Bleeding history: In total, the mean number of bleeding (SD) events during the last 12 months before the study was 22.8 ± 39.5. The numbers for sequence A and B were 27.8 ± 50.0 and 17.7 ± 19.8, respectively.

The Immunogenicity Phase began after the 30 hours PK blood sample. All bleedings were treated with AryoSeven. Menstruations were not counted as bleeding episodes (most of menstruation episodes have been treated with AryoSeven but they were not assessed for response to the treatment due to the difficulties in assessing response of a menstruation episode). During the Immunogenicity Phase there were 772 single administrations of AryoSeven for treatment on demand of 502 bleedings. Six subjects had no bleeding during the Immunogenicity Phase, three of them received prophylaxis of bleeding with AryoSeven.

Over 24 patients, during the 12 months of the Immunogenicity Phase there was a mean of 20.9 bleeding episodes per patient (Std: 30.07; Median: 7; Q1-Q3: 05 – 24; Min-Max: 0 – 100). Almost all 502 bleedings treated with AryoSeven (98%) completely resolved within 12 hours, as reported in Table 6 below. All patients resolved their bleeding. No cases of lack of efficacy have been reported.

Table 6: Bleeding Episodes during Immunogenicity Phase (Immunogenicity Analysis Set)

	Bleeding response n (%)
No bleeding episodes per patient:	
Number of patients	24
Mean	20.9
Std	30.07
Median	7.0
Q1-Q3	0.5-24
Min-Max	0-110
Total number of bleedings	502
Treatment response 2 h post-infusion:	
Non-responder	58 (11.6)
Responder	435 (86.7)
Unknown	9 (1.8)
Treatment response 6 h post-infusion:	
Non-responder	26 (5.2)
Responder	467 (93)
Unknown	9 (1.8)
Treatment response 12 h post-infusion:	
Non-responder	--
Responder	492 (98)
Unknown	10 (2.0)

Clinical efficacy with NovoSeven:

To assess the clinical response of NovoSeven for treating bleeding, publicly available information of NovoSeven was reviewed. The primary search was on NovoSeven resulting in 5561 references in PubMed. In most of the studies, patients received home treatment with patients who self-administered treatment and evaluated outcome.

Earlier studies are summarised in Table 11 (Hedner 2008).

Table 11. Results of home treatment of bleeding on demand with NovoSeven in patients with hemophilia A/B with inhibitor (Hedner 2008).

Authors	No. of patients	No. of bleedings	Dose (µg/kg)	Haemostasis (%)
Key 1998	60	614	90	92
Laurian 1998	16	147	90-120	90
Santagostino 2006[c]	21	53	90	79
Kavakli 2006	24	2/doses	270x1; 90x3	90 - 85
Young 2008	21	68	270x1; 90x3	91 - 90

Study	Subjects	No of bleeding	Dose	Efficacy measure	NovoSeven efficacy rates
Systematic review based on	Not indicated	Not indicated	90µg/kg every 2-3 hours or 1×270 µg/kg	Not indicated	81%-91% 12 hours after treatment initiation.

18 studies (Knight 2009)					
FENOC study (Astermark 2007)	66 patients enrolled (mean age 27.5 years, range 8 – 55 years) with haemophilia A with inhibitor	96 treated with aPCC or NovoSeven	Mean dose administered 212.5 µg/kg, range 98.6 – 261.8 µg/kg	4 items scale (effective, partially effective, poorly effective, or not effective)	60.4% after 2 h, 78.7% after 6h, 84.4% after 12 h, 85.7% after 24 h and 90.2% after 36 h (estimated power of 54%).
Haemophilia and Thrombosis Research Society registry (Young 2012, Neufeld 2011)	129 patients	2041	Not indicated	Not indicated	Young 2012: ranged from 89% in spontaneous bleeds to 93% in traumatic bleeds Neufeld 2011: 93% of bleeds overall
Bensadok 2017	27 patients enrolled (mean age 18.4±8.3 years, range 5-41 years) with congenital haemophilia and inhibitors	Not indicated	Not indicated	3 points scale: - effective, partially effective or ineffective with 'effective' or 'partially effective' responses considered as 'responders'.	87.8% at 9 h, with pain relief for 84.0%.
International ONE Registry (Chambost 2013)	85 patients with haemophilia A/B with inhibitor	494	≤120 µg/kg rFVIIan > 120 to <250 µg/kg rFVIIa and ≥250 µg/kg rFVIIa	Not indicated	At 9 h: 85% with ≤120 µg/kg rFVIIa , 96% with > 120 to <250 µg/kg rFVIIa and 86% with≥250 µg/kg rFVIIa.
Multinational, observational study SMART-7 (Kavakli 2017),	51 subjects with haemophilia A or B with inhibitors enrolled	538	Not indicated	Based on patient evaluations, treatment was described as "effective," "partially effective," or "ineffective," respectively.	94.2% stopped by end of treatment (NovoSeven® monotherapy)
Post hoc, subgroup analysis of SMART-7™ data (Demartis 2017)	45 subjects	482	Not indicated		96.5% within 1 hour after bleeding onset 93.1% between >1 to <4 hours after the onset

3.3.4.3. Clinical studies in special populations

Not applicable for biosimilars.

3.3.4.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

3.3.4.5. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

3.3.4.6. Supportive studies

Clinical efficacy was investigated in two supportive studies (**IRCT201202106302N1** and **IRCT201202106302N2**, see clinical AR) conducted for the approval of the drug in Iran. But the studies have several deficiencies and it is of doubt if the supportive studies should contribute to the efficacy assessment at all as they do not meet EU standards. Therefore, these studies are not considered relevant to back up the pivotal studies regarding efficacy.

3.3.5. Discussion on clinical efficacy

No dedicated efficacy studies were performed in patients. As only a weak PK-response relationship has been described for eptacog alfa and efficacy studies are unlikely to be sensitive enough to detect differences, the company has considered extended comparative PK/ PD characterisation for the demonstration of biosimilarity. Information on efficacy within these trials is considered supporting information. As already discussed during the EMA-SA (EMA/CHMP/SAWP/168901/2015), the usual considerations for an equivalence trial should be observed. However, the applicant mainly collected efficacy data in the immunogenicity follow-up period without a comparator.

The efficacy assessment is based on a 4 -oint rating scale that is a frequently used tool to measure bleeding response of treatment. Successful treatment was defined as an either “excellent” or “good” response. The investigator reviewed the at-home assessments of the patients during the control visits. The assessment of the endpoint was carried out at 2, 6 and 12 hours post-infusion. These time points are considered most sensitive to differences in efficacy between treatments according to previous studies comparing FEIBA and NovoSeven. The efficacy endpoint is therefore acceptable and allows assessment of bleeding control at different time points. However, it is not clear which time points were evaluated for efficacy, as the CSR of studies UGA 2014-01 and UGA 2014-02 state that response to treatment was assessed "at both time points". The applicant should therefore explain exactly which time points were assessed.

According to the study protocols (UGA 2014-01 and UGA 2014-02), treatment response was to be evaluated for multiple bleeding episodes using a generalised linear model for categorical data with repeated measurements in time. This was not performed in the study report and should be provided by the applicant. It is assumed that the proportion of responders and corresponding 95% CI will be estimated by the model at each protocol timepoint of response assessment. Missing (unknown) treatment response should be counted as non-response in this analysis.

In study **UGA 2014-01**, efficacy data are available for 42 patients. Six patients were at the beginning of the follow-up at the time of the database freezing for the statistical analysis, and consequently, there are no data on bleeding. Accordingly, the applicant should submit the missing data.

863 bleedings were treated with 2236 single administrations of AryoSeven, with a mean of 20.6 bleedings per patient. Almost all bleedings were completely resolved within 12 hours (829/863, 96%). The applicant mentions that the results are consistent with the success rate in controlling bleeding from previous NovoSeven studies. However, a direct comparison of the bleeding rates is missing and should be performed for UGA 2014-01.

In study **UGA 2014-02**, efficacy data are available for 24 patients.

There were 772 single administrations of AryoSeven for treatment on demand of 502 bleedings. Six subjects had no bleeding during the Immunogenicity Phase (3 of them received prophylaxis of bleeding with AryoSeven). Over 24 patients, during the 12 months of the Immunogenicity Phase there was a mean of 20.9 bleeding episodes per patient. Almost all 502 bleedings treated with AryoSeven (98%) completely resolved within 12 hours.

Three patients used anti-fibrinolytic medication while on study. Although this is not considered to interfere with FVII levels and thus not to affect the PK evaluation, anti-fibrinolytic drugs may bias the efficacy evaluation in the way that potential haemorrhagic events may be masked. The applicant should justify the concomitant use of anti-fibrinolytics in a substantial percentage of patients in study UGA 2014-02 with regard to interference with the efficacy evaluation.

According to the predefined methods, treatment should be assessed as failure if at least 3 doses of AryoSeven have been administered. However, no such data were presented in the Summary of Clinical Efficacy. The applicant is requested to provide the efficacy results as treatments per patient and bleeding episode.

A direct comparison of the bleeding rates to literature data for NovoSeven is missing and should be performed for UGA 2014-02.

3.3.6. Conclusions on clinical efficacy

Conclusions on comparable clinical efficacy cannot be drawn at this stage as patients are still in follow-up (UGA 2014-01) and the success rate in controlling bleeding has not been compared with the results of previous NovoSeven trials.

3.3.7. Clinical safety

The clinical development programme for HemAryo has included several clinical investigations for treatment of haemophilia A/B with inhibitors (UGA 2014-01), Factor VII deficiency (UGA 2014-02), two clinical trials conducted for the marketing authorisation of the product in Iran in patients with haemophilia A/B with inhibitors (IRTC201202106302N2) and in patients with Factor VII deficiency (IRTC201104266302N1), and an Iranian Post Marketing Surveillance study (ARY 2016-01).

Studies UGA 2014-01 and UGA 2014-02 are considered pivotal studies for the MAA of HemAryo and the main safety data were collected in the follow-up period of these studies. The reference product was only administered once during the PK cross-over phase and the single-arm design of the follow-up period without comparator makes a proper comparative assessment impossible in both studies.

The studies conducted for the marketing authorisation in Iran were designed for comparative analysis of safety and efficacy of AryoSeven with NovoSeven. However, due to the short duration of studies as well as notable deficiencies regarding EU GCP standards, the value of safety data from these short-term cross-over studies is limited.

3.3.7.1. Patient exposure

Clinical safety of HemAryo has been evaluated in 5 clinical studies: Study UGA 2014-01 (n=48 patients), UGA 2014-02 (n=24 patients), IRCT201202106302N1 (n=66 patients), IRCT201202106302N2 (n=66) and ARY2016-01 (n=214).

A total of 346 patients received at least one dose of HemAryo.

Table 8: Study Subject Drug Exposure

Study ID	UGA 2014-01	UGA 2014-02	IRCT201202106302N2	IRCT201202106302N1	ARY2016-01
Dose (µg/kg) [a]	90	30	90-120	30	-
No. of subjects	42	24	31	35	214
No of subjects with bleeding	42	19*	31	-	-
No of subjects in prophylaxis	23	9	-	35	-
Number of bleedings	863	502	31	NA	-
Mean per subject	20.6	20.9	1	NA	-
Total No. of doses	2236	772	93	105	-
Mean per bleeding	2.67	1.53	3	4[f]	-
Range [b]	1-58[c]	0-110[e]	-	NA	-
Length of treatment (days)					
Mean	2.67	1.53	6 hours	4 weeks	
Range [b]	1-22 [d]	-	NA	NA	-

[a] During the 12 months follow-up phase; [b] Including prophylaxis; [c] Data obtained from Table 14.3.2.1 of study 2014-01; [d] Data obtained from Listing 16.19.1 study UGA 2014-01; [e] Data obtained from Table 14.2.6 of study UGA 2014-02; [f] No. for doses per patient; * Menstruations are not counted as bleeding episodes (most of menstruation episodes have been treated with HemAryo but they were not assessed for response to the treatment due to the difficulties in assessing response of a menstruation episode

Across all studies, 3206 infusions of HemAryo were administered, representing 1396 bleeding episodes.

In study UGA 2014-01, the mean number of doses per bleeding was 2.67 with a median duration of 2.67 days for those receiving 90 µg/kg during the 12 months follow-up phase.

Table 9: Demographic Data

	UGA 2014-01 (n=42)	UGA 2014-02 (n=24)	ARY2016-01 (n=221)	IRCT201202106302 N2 (n = 31)	IRCT201202106302N 1 (n=35)
Age (years)					
Mean (SD)	27.6 (10.47)	25.0 (9.23)	23.8	20.5	21.8 (15.7)
Median	27.5	24	-	-	-
Range	12 - 56	12 - 45	5m-56	2-53	2.0-65.0
Age categories					
Pediatric	11 (22.9%)	3 (20%)	67 (30.3%)	30 (45.4%)	-
Adults	37 (71.1%)	19 (79.2%)	154 (69.7%)	33 (54.6%)	-
Gender					
Female	0	13 (54.2%)	99 (44.8%)	0	33 (50%)
Male	48 (100%)	11 (45.8%)	122 (55.2%)	66 (100%)	33 (50%)
Weight (kg)					
Mean (SD)	64.16 (14.25)	66.20 (17.22)			
Median	62.60	64.15			
Range	31.0-91.8	36.0-116.7			

The patients enrolled in the studies UGA 2014-01 and UGA 2014-02 were similar in most demographic variables, apart from gender. The major difference between the various studies was that UGA 2014-01 and UGA 2014-02 included a paediatric population of >12 years old, whereas the supportive studies IRCT201202106302N2 and IRCT201202106302N1 included a paediatric population of patients >2 years old and study ARY 2016-01 had no age limitation (including patients from 5 months old).

3.3.7.2. Adverse events

In studies UGA 2014-01 and UGA 2014-02, safety of HemAryo was assessed by monitoring adverse events (AEs), serious adverse events (SAE), clinical laboratory evaluations, vital signs, physical findings as well as immunogenicity. The adverse events (AEs) were coded using Medical Dictionary for Regulatory Activities (MedDRA, version 23.1).

Table 12. Adverse Events from Clinical Studies of HemAryo

	UGA 2014-01			UGA 2014-02			IRCT201202106302N2		IRCT201202106302N1	
	Cross-over phase		Immunogenicity phase	Cross-over phase		Immunogenicity phase	HemAryo (n=31)	NovoSeven (n=35)	HemAryo (n=35)	NovoSeven (n=31)
	HemAryo (n=48)	NovoSeven (n=48)	HemAryo (n=42)	HemAryo (n=24)	NovoSeven (n=24)	HemAryo (n=24)				
Any System Organ class Event										
- Subjects with at least one AE		*1*	*7*	*4*	*3*	*17*	*3*	*1*	*8*	*3*
- Total number of AEs		*1*	*12*	*4*	*4*	*39*	*3*	*1*	*12*	*4*
Adverse Events										
Blood and lymphatic system disorders										
Anaemia			1 (2.4%)	1 (4.2%)						
Gastrointestinal disorders										
Abdominal pain upper						2 (8.3%)				
Abdominal pain			1 (2.4%)	1 (4.2%)		2 (8.3%)				
Toothache						2 (8.3%)				
Vomiting			1 (2.4%)			1 (4.2%)				
Nausea						1 (4.2%)	1 (3.2%)		3 (8.57%)	
Diarrhoea						1 (4.2%)				
General disorders and administration site conditions										
Chest discomfort						1 (4.2%)				
Cyst						1 (4.2%)				
Fatigue						1 (4.2%)				
Fever									1 (2.85%)	
Local									1 (2.85%)	1 (3.22%)
Hepatobiliary disorders										
Hepatic steatosis			1 (2.4%)							
Infections and infestations										
Upper respiratory tract infection		1								
Nasopharyngitis	-		2 (4.8%)	-	1 (4.2%)	1 (4.2%)	-	-	-	-
Helicobacter gastritis	-		1 (2.4%)	1 (4.2%)	-	-	-	-	-	-
Tooth infection						1 (4.2%)				
Injury, poisoning and procedural complications										
Scratch						1 (4.2%)				
Investigations										
Haemoglobin decreased						4 (16.17%)				
Metabolism and nutrition disorders										
Hypoglycaemia			1 (2.4%)							
Vitamin B12 deficiency						1 (4.2%)				
Musculoskeletal and connective tissue disorders										
Muscle spasm										
Pain in extremities										
Pain in extremities										
Arthralgia									1 (2.85%)	
Haemarthrosis			1 (2.4%)							
Nervous system disorders										
Headache					1 (4.2%)	5 (20.8%)	1 (3.2%)	1 (2.9%)	3 (8.5%)	1 (3
Somnolence				1 (4.2%)						

Psychiatric disorders									
Insomnia					1 (4.2%)				
Respiratory, thoracic and mediastinal disorders									
Oropharyngeal pain					5 (20.8%)				
Renal and urinary disorders									
Dysuria	-	1 (2.4%)	-	-	-	-	-	-	-
Haematuria	-	1 (2.4%)	-	-	-	-	-	-	-
Renal pain	-	1 (2.4%)	-	-	-	-	-	-	-
Reproductive system and breast disorders									
Metrorrhagia					1 (4.2%)				
Skin and subcutaneous tissue disorders									
Rash						1 (3.2%)		1 (2.85%)	1 (3.22%)
Urticaria					1 (4.2%)			1 (2.85%)	1 (3.22%)
Surgical and medical procedures									
Gallbladder operation					1 (4.2%)				
Nasal operation					1 (4.2%)				
Vascular disorders									
Thrombophlebitis					1 (4.2%)				
Hypotension								1 (2.85%)	

Drug-related adverse events

Drug-related adverse events were those considered to be possibly, probably or definitely related to study treatment by the Investigator.

In study **UGA 2014-02**, there were two drug-related AE, both with HemAryo. One subject had pain in the extremity during the cross-over phase, which was considered moderate in the intensity and resolved completely after two days. Another subject had urticaria during the immunogenicity phase, which was considered attributable to HemAryo, moderate in the intensity and resolved completely after one day.

No drug-related adverse events occurred in study UGA 2014-01.

In the sole studies conducted with comparator, IRTC201104266302N1 and IRTC201104266302N2, AEs such as rash, local reaction, urticaria, fever, headache, hypotension, a.o., were frequently reported in the HemAryo group. According to the applicant, none of these potentially evocative signs of infusion-related reactions were evaluated by the investigators as possibly or probably related to study medications. Nevertheless, due to the several deficiencies of these studies, the relevance and the usefulness of safety data from these studies are doubtful.

3.3.7.3. Serious adverse events, deaths, and other significant events

In study **UGA 2014-01**, three AEs were considered as serious AEs (SAEs): hypoglycaemia, haemarthrosis and hematuria. According to the applicant, all three SAEs were considered not related and completely resolved except the hemarthrosis that resolved with sequelae.

No SAEs occurred in studies UGA 2014-02, IRTC201104266302N1 and IRTC201104266302N2.

No deaths were reported in the clinical trials.

3.3.7.4. Laboratory findings

The following clinical laboratory parameters have been evaluated for **UGA 2014-01** and **UGA 2014-02** at the local laboratories bevor treatment (visit 1), before the first product of the cross-over phase (visit 2), before the second product of the cross-over phase (visit 3) and then every 3 months for one year:

• Haematology: haemoglobin, haematocrit, red blood cells, white blood cell count with differential, platelet count

• Coagulation: PT, APTT, Fibrinogen, D-dimer, anti-thrombin activity

• Chemistry: blood urea nitrogen, serum creatinine, uric acid, bilirubin (total), lactate dehydrogenase (LDH), AST (SGOT), ALT (SGPT), Sodium, Calcium, Phosphate, Glucose, Albumin.

Laboratory and/or instrumental findings that were considered serious, whether expected or unexpected, and whether regarded as caused by the study drug, have been considered serious adverse events and had to be reported by the Investigator as soon as possible, and not later than 24 hours, to the Sponsor or its representative using the Serious Adverse Event Report forms.

In study **UGA 2014-01**, one subject had clinically significant low values of erythrocytes, haematocrit and haemoglobin at follow-up month 9 and clinically significant low values of haematocrit and haemoglobin at follow-up month 12. Four other subjects presented with clinically significant high values of glucose, lactate dehydrogenase or D-dimer.

Overall, no clinically significant abnormal values were reported in study **UGA 2014-02** apart from a high D-dimer value determined in one subject at month 9.

3.3.7.5. In vitro biomarker test for patient selection for safety

Not applicable

3.3.7.6. Safety in special populations

Not applicable for biosimilars

3.3.7.7. Immunological events

Development of neutralising antibodies (inhibitors) directed against coagulation factors (e.g. Factor VII, Factor VIII, Factor IX) is the most significant treatment complication in patients with haemophilia since it poses life-threatening risks and it decreases effectiveness of treatment. Development of accurate assays to measure inhibitors remains an essential requirement in the diagnosis and monitoring of treatment of patients with haemophilia. Thus the applicant was advised in both EMA-SAs to thoroughly assess HemAryo immunogenicity according to guideline EMEA/CHMP/BMWP/14327/2006. The Bethesda assay was proposed by the applicant to assess immunogenicity. In brief, the reduction of endogenous FVII activity is assessed by a prothrombin time assay performed in 50% pooled normal human plasma mixed with 50% plasma sample volume or a serial dilution of human FVII specific polyclonal goat IgG, in FVII depleted human plasma. The percentage of residual activity is calculated and depends on the amount of inhibitors in the sample. First, the Bethesda assay is not an immunogenicity assay. It is not specific for FVII, nor for an induced immune response. It recognises all kind of inhibitors raised against mostly all compounds of the human blood coagulation cascade. Second, presented assay is irrelevant for the assessment of the immunogenicity of the API. The recombinant rFVIIa molecule developed by the applicant differs considerably from the endogenous version, especially by exposing potentially immunogenic epitopes due to different post-translational modifications, aggregates and degradation products, like altered oligosaccharide structures, aggregates, oxidised forms, degraded forms, deamidated forms, and others like highly immunogenic impurities e.g. HCP, murine or bovine IgG contained in AryoGen DP. Immunogenic epitopes specific for the recombinant hFVIIa variant and its specific impurities are not present in - and not addressed by - the assay. Third, mentioned strategy is not in line with the concept proposed in the Guideline on Immunogenicity assessment of therapeutic proteins EMEA/CHMP/BMWP/14327/2006: No screening of

induced antibodies against baseline reactivity was performed at all, positivity of identified candidates was not checked by a confirmatory assay, and the neutralising potential was not assessed.

The applicant provided an assay validation protocol to be run at Research Toxicology Centre S.p.A. Italy. Validation activities and acceptance criteria for robustness, linearity, precision, and sensitivity were defined for this assay, but no validation report was submitted. A validation protocol and a validation report for a different activity assay, performed two years before at the Iranian blood transfusion organisation, were provided instead. Thus the applicant is asked to clarify which assay was used to assess clinical samples from studies performed in Europe, and to document validation activities performed for exactly the assay which was used to assess immunogenicity of respective clinical samples.

Immunogenicity has been evaluated in study **UGA 2014-01**, **UGA 2014-02** and **ARY 2016-01** in a total of 293 patients.

In study **UGA 2014-01** immunogenicity was assessed in all 48 patients at baseline, visit 1, visit 3 and in the immunogenicity phase every 3 months up to 1 year. Laboratory personnel at local and central labs assessing anti-FVIIa immunogenicity was blinded to the treatment received by the patient in the cross-over phase. All 48 patients who completed the PK/PD phase had immunogenicity negative at visit 3, before receiving the 2nd dose of the crossover phase.

As the immunogenicity follow-up is not complete, the study is still ongoing for 6 patients.

So far, none of the patients were positive for anti-drug antibodies.

Study 2014-02

The assessment of immunogenicity has been done in all 24 patients included in the PK phase, at baseline, visit 1, visit 3 and in the immunogenicity phase every 3 months up to 1 year (4 visits). Laboratory personnel at local and central labs assessing anti-FVIIa immunogenicity was blinded to the treatment received by the patient in the cross-over phase.

The immunogenicity (presence of neutralising antibodies against eptacog alfa) has been determined by the PT based modified Nijmegen method of the Bethesda assay, by the central lab and the centres in Turkey. The centres in Iran used the Bethesda assay. These patients samples have been retested by the central lab (using the Nijmegen modification) to confirm results.

All 24 patients, followed for 12 months, tested negative for anti-drug antibodies.

Study ARY2016-01 is discussed in detail under Post marketing experience.

3.3.7.8. Safety related to drug-drug interactions and other interactions

Not applicable for biosimilars.

3.3.7.9. Discontinuation due to adverse events

None of the AEs led to discontinuation of the study drug HemAryo.

3.3.7.10. Post marketing experience

Study **ARY2016-01** was a non-interventional, observational, prospective data collection of immunogenicity (neutralising antibodies toward rFVIIa) in patients who have received one or more dose of AryoSeven in the daily practice, for treatment or prophylaxis of bleeding of Haemophilia A and B with inhibitors, Factor VII Deficiency, Glanzmann's thrombasthenia or other uses. Patients have been be prospectively blood tested for immunogenicity. The study has been conducted in Iran where

AryoSeven is available on the market since 21-08-2012 and Novoseven is no longer available since 2013.

Immunogenicity has been tested at screening and follow-up visit by a centralised laboratory in Iran (the Iranian Blood Transfusion Organization/IBTO) using the PT-based Bethesda Assay. In case of positive results for inhibitor, an inhibitor retest using a separately drawn sample as confirmatory measurement was performed by IBTO and a sample of patient's plasma sent to an external, independent, reference centre for confirmation (Dept. of Hematology of University of Milano, Italy).

According to the clinical study report of study ARY2016-01, immunogenicity assessment (neutralising FVII antibodies) has been conducted by the study central lab (IBTO) using the PT-based Bethesda Assay method. No detailed description of the methodology to determine anti-drug antibodies has been provided in the CSR. A major objection has been raised on the use of the Bethesda assay for the evaluation of immunogenicity towards FVIIa in the pivotal studies UGA 2014-01 and UGA 2014-02 (see Section 2.1.2 of this report). The applicant is requested to specify whether the same method was used in ARY2016-01 or provide a detailed assay description in case a different methodology has been applied. (**OC**)

Two patients without previous AryoSeven treatment were excluded from the study. This approach is not comprehensible, since previously untreated subjects are considered the most sensitive for development of anti-drug antibodies. Furthermore, it has not been justified why subjects positive for ADA at the screening visit were not included in the prospective part. Following-up on these patients might have provided valuable information about a potential change in ADA levels over time.

The following immunogenicity data have to be regarded with caution as their validity is not certain until the MO on the immunogenicity assay has been resolved.

The total prevalence of inhibitors attributable to AryoSeven was 1.4% (3/221 patients). Two of these three patients had severe congenital FVII deficiency (2.3%, 2/87 patients) and one had Glanzmann's thrombasthenia (1.2%, 1/81 patients). No patients with Haemophilia A (34 patients) had immunogenicity against rFVIIa. The total incidence of inhibitor development (de-novo cases) in the prospective analysis was 0.9% (2/214 patients). One of these patients had severe congenital FVII deficiency diagnosed at age of 2 months, and was 5 months old at time of screening. He had 6 months follow-up (between the screening and follow-up visit) with 63 exposure days (ED) during the prospective phase and 78 ED since the first use of AryoSeven. Bethesda results were 7.1 BU at follow-up visit and 4.1 BU at the retest after 3 months. The other patient was a 32-year-old female with Glanzmann's thrombasthenia, diagnosed at the age of 3 months. She had 25 months of follow-up with 32 ED during the prospective phase and 52 ED since the first use of AryoSeven. Bethesda results were 1.94 BU at the follow-up visit and 2.44 BU at the retest after 4 months.

According to the SmPC of the originator, there have been no reports of inhibitory antibodies against NovoSeven or FVII in patients with haemophilia A or B in post-marketing experience. Development of inhibitory antibodies to NovoSeven has been reported in a post-marketing observational registry of patients with congenital FVII deficiency. In clinical trials of patients with factor VII deficiency, formation of antibodies against NovoSeven and FVII is the only adverse drug reaction reported (frequency: common (≥ 1/100 to < 1/10)). In some cases, the antibodies showed inhibitory effect *in vitro*. Risk factors that may have contributed to antibody development including previous treatment with human plasma and/or plasma-derived factor VII, severe mutation of FVII gene, and overdose of NovoSeven, were present.

According to available literature, inhibitor development against FVII is a relatively rare phenomenon seen mainly in severe FVII deficiency, with 27 out of 380 patients (7.1%) developing inhibitors across

13 studies (summarised in a review by Ramezanpour et al. 2021). The inhibitor level observed in study ARY2016-01 is distinctly lower compared to these literature meta-data.

However, in order to relate the results of study ARY2016-01 to literature data, comparability of patient populations (especially with regard to previous exposure to rFVIIa) and the assays used to detect antibodies against FVII (especially the quality with which the analyses were performed) needs to be considered (see also the discussion in section 4.8 of this report).

In total, 14 patients (6.4%) reported any adverse events; the most common were vertigo (5), headache (3) and dyspnoea (2).

Four patients died during the course of the study. Narratives of all deaths were provided. None of these cases was considered drug related.

In total, 8 patients (3.7%) had adverse events which were considered drug-related. Seven of those were not considered serious AEs (3 cases of dyspnoea, 2 cases of headache, and 1 case each of arthralgia and injection site reaction).

One drug related SAE of deep vein thrombosis was observed in a patient with acquired FVII deficiency. This patient received HemAryo treatment because she was undergoing surgery and was treated for 21 days continuously. The deep vein thrombosis occurred 3 days after the last HemAryo administration. In general, thrombosis has been reported in FVII deficient patients receiving NovoSeven during surgery and is thus not considered an unexpected event in this study. However, according to the information provided in Module 2.7.4, this event was not resolved. The applicant is asked to specify how this event was followed up and if it eventually was resolved.

Overall, the results of post marketing study ARY2016-01 do not raise concerns for the use of HemAryo in the intended patient populations, but the data need to be regarded with caution due to methodological uncertainties pertaining to the assay method and the comparability to external data obtained with the reference product (see Discussion on Clinical Safety).

3.3.8. Discussion on clinical safety

The clinical development programme for HemAryo has included several clinical investigations for treatment of haemophilia A/B with inhibitors (UGA 2014-01), factor VII deficiency (UGA 2014-02), two clinical trials conducted for the marketing authorisation of the product in Iran in patients with haemophilia A/B with inhibitors (IRTC201202106302N2) and in patients with factor VII deficiency (IRTC201104266302N1), and an Iranian Post Marketing Surveillance study (ARY 2016-01).

Studies UGA 2014-01 and UGA 2014-02 are considered pivotal studies for the MAA of HemAryo, whereas the studies conducted for the marketing authorisation in Iran have several deficiencies in study design and contribute to the safety assessment in a supportive manner only.

Both indications used in the pivotal studies (haemophilia A or B with inhibitors and patients with congenital Factor VII deficiency) are considered as sufficiently sensitive populations to investigate clinical biosimilarity in terms of safety.

A total of 346 patients received at least one dose of HemAryo, including 66 in the pivotal studies, 66 in the supportive studies, and 214 in the post marketing study.

The majority of data was established in single-arm settings and only limited comparative safety data were provided. However, due to the rarity of the indications applied for and this being a submission for a biosimilar product, the scope of the provided safety data is acceptable.

The safety assessment conducted in the pivotal studies is overall considered appropriate, including coding and definition of adverse events, causality assessment, frequency of visits and follow-up duration.

In study **UGA 2014-01**, three SAEs were reported that were not related to HemAryo. In total, 13 AEs by 7 patients (16.67%) were reported, none of which were considered drug-related. None of these events led to discontinuation of the study drug.

Most haematology and biochemistry laboratory parameters evaluated during the clinical studies of HemAryo showed no apparent trends or clinically significant changes, other than those expected based on the pharmacologic action of the study drug.

Subject 100205 had clinically significant high values of glucose at baseline and follow-up month 3, subject 200301 had a clinically significant high value of lactate dehydrogenase at the visit of first study drug administration and Subject 100203 had high values of D-dimer at baseline and at both visits of study drug administration. Since these patients already had high values before treatment, it is excluded that HemAryo is responsible for the parameter shifts. The applicant is asked to provide more information on subject 200401 who experienced low values of haematocrit and haemoglobin at the last follow-up visit.

Since this study is still ongoing, safety (and immunogenicity) data should be submitted for the remaining 6 patients who are still in follow-up.

In study **UGA 2014-02**, 9 AEs by 6 subjects (25%) were reported during the cross-over phase and only one of them was considered drug-related. Additionally, 39 AEs by 17 subjects (70.8%) were reported during the immunogenicity phase and only one of them was considered drug-related. The drug-related AEs were both detected upon treatment with HemAryo. The events related to pain in the extremity and urticaria, respectively. Both were considered moderate in the intensity and resolved completely after one and two days, respectively. No serious AEs and no AEs leading to study discontinuation were reported in study UGA 2014-02.

The applicant is asked to provide more details of the case of pain in the extremity observed upon treatment with HemAryo – especially if this patient had taken any concomitant medication while on study and how his AE was resolved.

Further, a high D-dimer value was determined in one subject at month 9. The applicant is asked to provide more information on this laboratory parameter for the respective subject over the course of the study.

It is noticed that in each of the pivotal trials, the reported mean number of doses per bleeding is equal to the mean treatment duration. The applicant should verify the accuracy of these numbers.

In the supportive studies IRTC201104266302N1 and IRTC201104266302N2 all observed AEs were mild and considered not drug-related. Therefore, although these studies show a trend towards more frequent AEs in the HemAryo arm compared to the NovoSeven arm, no safety concern would arise from these data. In light of apparent deficiencies in study design, their relevance for clinical safety assessment remains questionable.

In the post marketing study **ARY2016-01**, one drug related SAE of deep vein thrombosis was observed in a patient with acquired FVII deficiency undergoing surgery. In general, thrombosis has been reported in FVII deficient patients receiving NovoSeven during surgery and is thus not considered an unexpected event in this study. However, according to the information provided in Module 2.7.4, this event was not resolved. The applicant is asked to specify how this event was followed up and if it eventually was resolved.

Overall, no specific pattern can be identified from the analysis of AEs across all studies conducted and the observed adverse events reflect the safety profile of the reference product NovoSeven.

Immunogenicity

A major objection is raised on the immunogenicity assay used to determine anti-drug antibodies to FVIIa. First, the Bethesda assay is not an immunogenicity assay. It is not specific for FVII, nor for an induced immune response. It recognises **all** kind of inhibitors raised against mostly **all** compounds of the human blood coagulation cascade. Second, presented assay is irrelevant for the assessment of the immunogenicity of the API. The recombinant rFVIIa molecule developed by the applicant differs considerably from the endogenous version, especially by exposing potentially immunogenic epitopes due to different post-translational modifications, aggregates and degradation products, like altered oligosaccharide structures, aggregates, oxidised forms, degraded forms, de-amidated forms, and others. All epitopes specific for the recombinant FVIIa variant are not present in - and not addressed by - the assay. Third, mentioned strategy is not in line with the concept proposed in the Guideline on Immunogenicity assessment of therapeutic proteins EMEA/CHMP/BMWP/14327/2006: No screening of induced antibodies against baseline reactivity was performed at all, positivity of identified candidates was not checked by a confirmatory assay, and the neutralising potential was not assessed. Thus, the applicant is required to justify the absence of a multi-tiered approach for immunogenicity assessment and to discuss how immunogenicity of the API and especially of an immune response raised against product and process related impurities should be evaluated based on the proposed assay.

The applicant provided an assay validation protocol for a modified version of the Bethesda assay, to be run at Research Toxicology Centre S.p.A. Italy. This assay is a FVII activity assay which was proposed instead of an immunogenicity assay. Validation activities and acceptance criteria for robustness, linearity, precision, and sensitivity were defined for this assay, but no validation report was submitted. A validation protocol and a validation report for the former, not modified Bethesda assay, performed two years before at the Iranian blood transfusion organisation, were provided instead. The applicant is asked to clarify which assay was used to assess clinical samples of studies performed in Europe, and to document validation activities performed for exactly the assay which was used to assess immunogenicity of respective clinical samples.

Thus the presented immunogenicity data have to be regarded with caution as their validity is not certain.

Anti-drug antibodies have not been observed in the pivotal studies UGA 2014-01 and 2014-02.

Immunogenicity towards exogenous FVII protein is rarely seen in Haemophilia patients treated with FVIIa as a bypassing agent but is not uncommon in FVII deficient patients, since the latter have very low levels of endogenous FVII protein. Thus, the population most sensitive to FVIIa treatment is congenital FVII deficiency without previous exposure to FVII(a) drug products.

According to the inclusion and exclusion criteria of studies UGA 2014-01 and 2014-02, previous treatment with AryoSeven or NovoSeven was allowed whereas subjects with pre-existing antibodies against FVII were to be excluded. This renders the immunogenicity assessment of patients included in the PK and PK/PD groups of the pivotal studies futile.

It has been concluded during the scientific advice procedures for HemAryo that immunogenicity would be assessed in FVII deficient patients with no or only few (1-5) previous FVIIa treatments. For this purpose, it was planned to enrol a separate patient group in study UGA 2014-02. It was also agreed that this assessment could start pre-authorisation and continue post-authorisation. However, this part of the study was not carried out, which is considered a major protocol deviation.

Additionally, the applicant performed a post marketing immunogenicity study (ARY2016-01) in Iran. According to the results of this study the total prevalence of inhibitors attributable to AryoSeven was 1.4% (3/221 patients) and thus lower than what has been reported for NovoSeven in literature. However, these data need to be regarded with caution based on deficiencies regarding assay method and exclusion criteria. Furthermore, comparability of patient populations and the assays used to detect ADAs between this study and external data needs to be discussed.

It is also noted that inhibitory antibodies were detected in a patient with Glanzmann's thrombasthenia in study ARY2016-01. Development of inhibitory antibodies to rFVIIa in this disease are extremely rare.

Taking into account the above listed issues and on top of the requirement to resolve the major objection related to the immunogenicity assay, the following concern is raised regarding the immunogenicity assessment of HemAryo:

a) The applicant should give a comprehensive explanation why the group for immunogenicity assessment was not included in study 2014-02.

b) The applicant should justify the exclusion of previously untreated patients from study ARY2016-01 and the exclusion of patients with ADA present at baseline from the prospective part of that study.

c) The applicant should provide more information on the case of a patient with Glanzmann's thrombasthenia who developed inhibitory antibodies to rFVIIa in order to confirm that the patient indeed had Glanzmann's thrombasthenia, if this patient possibly also had FVII deficiency and that indeed an inhibiting antibody was detected.

d) The applicant should discuss comparability of patient populations (especially with regard to previous exposure to rFVIIa) and the assays used to detect antibodies against FVII (especially the quality with which the analyses were performed) in order to relate the results of study ARY2016-01 to literature data.

e) The applicant should provide data on patients previously treated with AryoSeven in Iran who stopped treatment due to inhibitor formation or hypersensitivity reactions.

f) The applicant should provide an integrated immunogenicity assessment taking into account the above listed points and elaborate on plans to potentially evaluate immunogenicity of HemAryo in future studies.

3.3.9. Conclusions on clinical safety

Overall, no specific pattern can be identified from the analysis of AEs across all studies conducted and the observed adverse events reflect the safety profile of the reference product NovoSeven. However, several methodological deficiencies preclude the assessment of immunogenicity, including a major objection on the immunogenicity assay used.

3.4. Risk management plan

3.4.1. Safety Specification

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Venous thromboembolic events Arterial thromboembolic events Immunogenicity (FVII deficiency)
Important potential risks	Immunogenicity (potential risk for acquired haemophilia, congenital haemophilia A and B, Glanzmann's thrombasthenia) Medication errors
Missing information	Elderly patients Pregnant and lactating women Single high-dose use (270 µg/kg; in haemophilia A and B with inhibitors)

Recommendations of the CHMP Rapporteur's D80 overview & Co-Rapporteur's D96 Critique AR

In line with the requirements for biosimilar products, the summary of safety concerns corresponds to the most recent RMP of the originator product.

However, there is an additional risk of "medication errors" as the reconstituted solution of the comparator product has a concentration of 1mg/ml and that of the biosimilar has a concentration of 0.6 mg/ml. Since the strength of the originator is higher, more ml of rFVIIa biosimilar are needed for the same dose. Therefore, it is endorsed that medication error is listed as an important potential risk in the summary of safety concerns.

Additional RMMs should be considered.

Having considered the data in the safety specification, the rapporteur agrees that the safety concerns listed by the applicant are appropriate.

3.4.1.1. Discussion on safety specification

In line with the requirements for biosimilar products, the summary of safety concerns corresponds to the most recent RMP of the originator product.

However, there is an additional risk of "medication errors" as the reconstituted solution of the comparator product has a concentration of 1mg/ml and that of the biosimilar has a concentration of 0.6 mg/ml. Since the strength of the originator is higher, more ml of rFVIIa biosimilar are needed for the same dose. Therefore, it is endorsed that medication error is listed as an important potential risk in the summary of safety concerns.

Additional RMMs should be considered.

3.4.1.2. Conclusions on the safety specification

Having considered the data in the safety specification, it is agreed that the safety concerns listed by the applicant are appropriate.

3.4.2. Pharmacovigilance plan

Routine pharmacovigilance activities

There are no routine pharmacovigilance activities beyond adverse reactions reporting and signal detection.

PRAC Rapporteur assessor's comment:

The reference product Novoseven has two specific adverse reaction follow-up questionnaires for the safety concerns immunogenicity and thromboembolic events.

Immunogenicity:

If inhibitors are suspected, a structured follow-up form for immunogenicity will be used to ascertain additional information. The questionnaire includes questions on treatment, history of inhibitors and whether the reporters would like assistance to test for antibodies to eptacog alfa. The form also documents relevant laboratory tests performed.

Thromboembolic events:

If embolic or thrombotic events are reported, structured follow-up forms with questions related to the handling of the product regarding the time from reconstitution to administration will be used. The objective is to assess the potential risk of embolic and thrombotic events associated with rFXIII.

Therefore, the applicant is requested to include two specific adverse reaction follow-up questionnaires for the safety concerns immunogenicity and thromboembolic events in line with Novoseven in part III and Annex 4 of the RMP.

Additional pharmacovigilance activities

Not applicable. For the planned post-authorisation safety studies please see Table Part III.3.1 below.

Summary of planned additional PhV activities from RMP

Table Part III.3.1: On-going and planned additional pharmacovigilance activities

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 – Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
UGA 2014-01, A randomised, multicentre, double blind clinical trial comparing pharmacodynamic, pharmacokinetic and safety of a biosimilar eptacog alfa (Aryoseven) and Novoseven®, in patients with heamophilia A or B with inhibitors Ongoing	Primary objectives: - To demonstrate bioequivalence of biosimilar eptacog alfa compared with NovoSeven, based on primary pharmacokinetic and pharmacodynamic parameters. Secondary objectives: - To demonstrate similar pharmacokinetic profile of the biosimilar eptacog alfa and NovoSeven® with regards to other PK parameters. - Clinical response in controlling acute bleeding.	Formation of neutralising Antibodies Adverse events	Protocol Last patient out Final study report	18/02/2020 31/10/2021 31/12/2021

	Safety - To evaluate immunogenicity of biosimilar eptacog alfa (formation of neutralising antibodies) - Adverse Events			

PRAC Rapporteur assessor's comment:

The applicant included the ongoing study UGA 2014-01 (clinical trial comparing pharmacodynamic, pharmacokinetic and safety of biosimilar) as a category 1 additional pharmacovigilance activity. The pharmacovigilance plan should include the additional pharmacovigilance activities for further identification or characterisation of specific safety concerns listed in the summary of safety concerns. Since this is not the case for study UGA 2014-01, this study should be removed from the pharmacovigilance plan. NovoSeven has no ongoing or planned additional pharmacovigilance studies/activities (imposed, mandatory or required [Category 1–3]) (RMP version 7.1, dated 16 Nov 2018).

Plans for additional pharmacovigilance activities are pending the response to the list of questions by the CHMP Rapporteur.

Overall conclusions on the PhV Plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

Plans for post-authorisation efficacy studies

Summary of Post authorisation efficacy development plan

Table Part IV.1: Planned and on-going post-authorisation efficacy studies that are conditions of the marketing authorisation or that are specific obligations.

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due Date
Efficacy studies which are conditions of the marketing authorisation				

UGA 2020-01 An exploratory study to evaluate the dose response relationship of pharmacodynamic parameters of AryoSeven in patients with haemophilia A or B with inhibitors UGA 2020-01 Ongoing	Primary objective: - To demonstrate a dose-response relationship of AryoSeven dose and TGA (lag-time) Secondary objectives: - To explore a dose-response relationship of AryoSeven dose and TGA (other parameters, such as peak height, ETP) - To explore a dose-response relationship of AryoSeven dose and D-dimer plasma concentration - To explore a dose-response relationship of Aryoseven dose and F1-2 plasma concentration - To explore PK/PD relationship - To explore the difference in PD parameters of AryoSeven and NovoSeven	Dose-response or concentration response relationship for PD markers (TGA, D-dimer, F1.2), This study includes 4 different doses of AryoSeven and 1 dose of Novoseven.	Protocol Final report	22-10-2020 15-09-2021
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				

PRAC Rapporteur assessor's comment:

The MAH proposed a PAES as a condition of the marketing authorisation, UGA 2020-01, an ongoing exploratory study to evaluate the dose response relationship of pharmacodynamic parameters of AryoSeven in patients with haemophilia A or B with inhibitors. This UGA 2020-01 study is an ongoing study which is part of the clinical development programme and is not considered a PAES. This study should be removed from the RMP section 'plans for post-authorisation efficacy studies'. Of note, NovoSeven has no ongoing or planned post-authorisation efficacy studies (RMP version 7.1, dated 16 Nov 2018).

The need of a PAES will be raised by the CHMP Rapporteur.

3.4.3. Risk minimisation measures

Routine Risk Minimisation Measures

Table Part V.1: Description of routine risk minimisation measures by safety concern

Safety concern	Routine risk minimisation activities
Venous thromboembolic events	Routine risk communication: *SmPC section 4.8* *PL section 2 and 4* Routine risk minimisation activities recommending specific clinical measures to address the risk: *SmPC Section 4.4 describes to weigh benefit of treatment against risk.* Other routine risk

	minimisation measures beyond the Product Information: *None*
Arterial thromboembolic events	Routine risk communication: *SmPC section 4.8* *PL section 2 and 4* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *SmPC Section 4.4 describes to weigh benefit of treatment against risk.* Other routine risk minimisation measures beyond the Product Information: *None*
Immunogenicity (FVII deficiency)	Routine risk communication: *SmPC section 4.8* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *SmPC Section 4.4 describes how treatment should be given in case of severebleeds.* *SmPC Section 4.4 describes precautions for suspected antibody formation.* Other routine risk minimisation measures beyond the Product Information: *None*
Medication errors	Routine risk communication: *SmPC section 4.2* *PL section 2* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *None* Other routine risk minimisation measures beyond the Product Information: *None*

Immunogenicity (acquired haemophilia, congenital haemophilia A andB, Glanzmann's thrombasthenia)	Routine risk communication: *SmPC section 4.8* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *SmPC Section 4.4 describes how treatment should be given in case of severebleeds.* Other routine risk minimisation measures beyond the Product Information: *None*
Elderly	Routine risk communication: *SmPC section 4.2* *PL section 2* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *None* Other routine risk minimisation measures beyond the Product Information: *None*
Pregnant and lactating women	Routine risk communication: *SmPC section 4.6* *PL section 2* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *None* Other routine risk minimisation measures beyond the Product Information: *None*
Single high-doseuse (270 µg/kg;in haemophilia Aand B with inhibitors)	Routine risk communication: *SmPC section 4.2* *PL section 2* Routine risk minimisation activities recommending specific clinical measuresto address the risk: *SmPC Section 4.4 describes how treatment should be given in case of severebleeds.* Other routine risk minimisation measures beyond the Product Information: *None*

Medication error:

SVII.3.1. Presentation of important identified risks and important potential risks

Medication errors (medication errors)	
Potential mechanism	The strength of rFVIIa is different from the strength of the comparator product NovoSeven, so there is a theoretical risk of medication errors. If prescribers/caregivers use the dose of the other product, the dose will be too low. medication errors theoretically may occur. If patients get a too highdose, no serious concerns are expected, but if patients get a too low dose, there may be therapeutic loss.
Evidence source(s) and strength of evidence	In clinical trials, no medication errors were reported, and no patients gotthe wrong strength. For two studies, a weight-dose converting table wasused.
Characterisation of the risk	The strength of the originator is higher, so for the same dose, more ml of rFVIIa biosimilar is needed. Prescribers and later patients have to use the weight-dose table. If mistakes are made, the strength may be not optimal.A too high strength was not toxic in the studies. However, a too low strength may cause unnecessary bleedings. Example: a patient weighing 70 kg needs 6.3 mL of NovoSeven®, and 10.5mL of rFVIIa for a dose of 90 µg/kg.
Risk factors and risk groups	Patients who used the other product may be at risk. New patients are notexpected to be at risk.
Preventability	The SmPC is clear on dosing instructions.
Impact on the risk-benefit balance of the product	Patients who get too low a dose, may suffer from unnecessary bleeding. Asno such events have occurred in the studies without table, it is expected that this impact will be negligible.
Public health impact	No impact is expected on public health.

PRAC Rapporteur assessor's comment:

Routine risk minimisation activities are considered sufficient to manage the safety concerns in line with NovoSeven (RMP version 7.1, dated 16 Nov 2018). The applicant identifies medication error as a theoretical risk and considers routine risk minimisation measures sufficient to manage this. However, to determine if routine risk minimisation measures are sufficient, more information is necessary. The applicant should provide information on the difference with NovoSeven regarding the colour, shape and size of the package, container, cap and vials (white powder and solvent). Furthermore, it is unclear if the risk minimisation materials used in the clinical studies are comparable to the routine risk minimisation materials (i.e. product information) used in post-marketing setting. The applicant should provide a discussion on the representativeness of the RCT setting and the routine risk minimisation measures used post-marketing.

Additional risk minimisation measures

Routine risk minimisation activities are sufficient to manage the safety concerns of the medicinal product.

PRAC Rapporteur assessor's comment:

Routine risk minimisation activities are considered sufficient to manage the safety concerns in line with NovoSeven (RMP version 7.1, dated 16 Nov 2018). However, the assessment of the risk minimisation measures of medication errors is pending the response to the list of questions.

Overall conclusions on risk minimisation measures

The PRAC Rapporteur, having considered the data submitted, is of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s), however, the applicant should provide supplementary information (see section 9. List of Questions).

3.4.4. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.1, date of final sign-off 16 June 2021, could be acceptable if the applicant implements the changes to the RMP as detailed in the endorsed Rapporteur assessment report and in the list of questions in section 6.3.

3.5. Pharmacovigilance

3.5.1. Pharmacovigilance system

It is considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.>

3.5.2. Periodic Safety Update Reports submission requirements

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

4. Non-Conformity with agreed Paediatric Investigation Plan

Not applicable for biosimilars.

5. Biosimilarity assessment

5.1. Comparability exercise and indications claimed

HemAryo (also referred to as AryoSeven) is developed as a biosimilar to the reference medicinal product (RMP) NovoSeven. The administration route (intravenous), posology, and indications are according to the RMP as described in the NovoSeven SmPC.

The marketing authorisation is claimed for the treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups:

• in patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX >5 Bethesda Units (BU)

• in patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration

• in patients with acquired haemophilia

• in patients with congenital FVII deficiency

• in patients with Glanzmann's thrombasthenia with past or present refractoriness to platelet transfusions, or where platelets are not readily available.

The RMP NovoSeven was introduced to the European market in 1996. NovoSeven was initially provided as a cold storage freeze-dried formulation (1.2 mg/vial presentation). Since 2008, a new NovoSeven formulation was introduced to the European market which differs in formulation and in active substance amount per vial (from 1.2 mg to 1.0 mg). Due to patent restrictions on the new formulation, the HemAyro drug product has been developed with reference to the old NovoSeven formulation, which is not on the market anymore. Due to difficulties with availability of the old 1.2 mg presentation of RMP the applicant used the 1 mg/vial presentation of RMP with the new formulation which is currently on the market for the conduct of the biosimilarity exercise.

At quality level biosimilarity evaluation was performed as a head-to-head comparison between NovoSeven (in the current marketed formulation) and HemAryo. Five batches of each NovoSeven (only three EU-batches) and HemAryo were used for these studies giving a five versus five head-to-head set up for each test. Physicochemical quality including primary and higher order structure, post-translational modifications, molecular weight and size, purity, product-related variants and impurities as well as certain biological activities were compared. In addition, forced degradation under stress conditions was performed to compare degradation pathways and kinetics.

Summary of clinical data

The clinical developmental programme comprises five clinical studies:

- **UGA 2014-01** was a Phase III, multicentre, randomised, double-blind, two-dose, two-ways cross-over bioequivalence clinical trial to compare the PK and PD of HemAryo and NovoSeven after a single iv. injection in 48 adult and paediatric patients (>12 years) with haemophilia A or B with inhibitors titer >5 Bethesda Units [BU] and with >2 episodes of bleeding requiring treatment with FVII infusions within the previous 12 months at trial start, not in bleeding status.
- **UGA 2014-02** was a multicentre, randomised, single dose, double blinded, two-way cross-over study, evaluating the pharmacokinetic of biosimilar eptacog alfa (AryoSeven) and NovoSeven (Novo Nordisk) in adult and paediatric (>12 years) patients with a confirmed diagnosis of severe congenital Factor VII Deficiency (FVII <1%), with >2 episodes of bleeding/year requiring treatment with FVII infusions.
- The **ARY 2016-01** was a non-interventional, observational, prospective data collection of immunogenicity (neutralising antibodies toward rFVIIa) in patients who have received one or more dose of AryoSeven in the daily practice for treatment or prophylaxis of bleeding of Haemophilia A and B with inhibitors, Factor VII Deficiency, Glanzmann's thrombasthenia or other uses.
- In addition, clinical efficacy was investigated in two supportive studies (**IRCT201202106302N1** and **IRCT201202106302N2**) conducted for the approval of the drug in Iran. But the studies have several deficiencies and it is of doubt if the supportive study

should contribute to the efficacy assessment at all as they do not meet EU standards. Therefore, these studies are not considered relevant to back up the pivotal studies regarding efficacy or safety.

The clinical development plan for HemAryo was aligned with the EMA guidelines CHMP/437/04 Rev1 and EMEA/CHMP/BMWP/14327/2006 and discussed with EMA through SA (EMA/CHMP/SAWP/168901/2015 and EMA/CHMP/SAWP/590140/2016).

5.2. Results supporting biosimilarity

At least for the main physicochemical quality attributes no major differences were observed. Also the comparative stress studies did not indicate significant differences in the degradation behaviour between RMP and biosimilar development. However, the validity of the available results is limited due to the major deficiencies as elaborated in section 5.3 below.

Clinical

Pharmacokinetics

The PK profiles of HemAryo and EU NovoSeven were both considered bioequivalent in terms of the primary endpoints as the 90% CI for the geometric LS means ratios were fully contained within the predefined bioequivalence limits of 0.80 to 1.25. Secondary endpoints were also in support of PK similarity.

Efficacy

Efficacy was assessed during the 12 months follow-up phase in the two pivotal clinical studies.

Currently efficacy data are available for 42 of 48 haemophilia patients in study UGA 2014-01. In this study 863 bleedings were treated with 2236 single administrations of AryoSeven, with a mean of 20.6 bleedings per patient. Almost all bleedings were completely resolved within 12 hours (96%).

Across the 24 patients with FVII deficiency in study UGA 2014-02, during the 12 months of the Immunogenicity Phase there was a mean of 20.9 bleeding episodes per patient. Almost all 502 bleedings treated with AryoSeven (98%) completely resolved within 12 hours.

Safety

In general, the majority of adverse events were mild to moderate in intensity and were not considered drug-related. Serious adverse events were only encountered in one of the pivotal studies and in the post marketing immunogenicity study. The SAEs in the pivotal study were not considered drug-related.

No deaths or adverse events leading to study discontinuation were encountered in any of the studies.

Overall, the observed adverse events reflect the spectrum of those described for the reference product, NovoSeven.

Immunogenicity

According to the results of a post marketing immunogenicity study (ARY2016-01) the total prevalence of inhibitors attributable to AryoSeven was 1.4% (3/221 patients) and thus lower than what has been reported for NovoSeven in literature. However, these data need to be regarded with caution based on deficiencies regarding assay method and exclusion criteria.

5.3. Uncertainties and limitations about biosimilarity

The presented biosimilarity exercise is far away from what is usually expected for a large, recombinant protein with multifaceted post-translational modifications and a complex mode of action. Several major deficiencies have been identified which currently preclude a biosimilarity claim of HemAryo to NovoSeven:

- The inclusion of only five RMP batches into the whole biosimilarity evaluation is far too limited to give an impression on the variability of the RMP on the market. As outlined in the EMA "Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)" multiple different batches of the reference medicinal product should be used to provide robust comparability data in order to generate a representative quality profile.
- In addition, the number of HemAryo batches included in the biosimilarity exercise is quite limited considering the long manufacturing history of HemAryo. The selection of the five HemAyro batches has not been justified and its remains unclear if a predefined sampling strategy has been in place. It seems that the currently selected HemAryo batches represent batches used in the clinical studies, but neither the full set of clinical batches nor process validation batches have been included for biosimilarity evaluation.
- No criticality assessment of the quality attributes of the rhFVIIa molecule has been presented. Without this essential information, it remains difficult to conclude if certain differences have to be considered relevant or not. In fact, certain differences such as higher oxidation, deamidation and hydroxylation levels for HemAryo have been observed, but not further discussed. It remains unclear if these differences have any impact on the efficacy and safety profile of the product.
- It remains completely unclear how and if at all similarity ranges for quantitative quality attributes have been established and on which criteria similarity has been concluded.
- For comparative characterisation of the biological functions only two assays – an SPR binding assay to tissue factor and a coagulation time assay were used. Considering the complex mode of action for rhFVIIa and its multifaceted interaction with different molecules during the blood coagulation, the set of two proposed assays is too limited to gain a full picture of the biological activities of rhFVIIa.
- No qualification/validation reports for the used methods have been provided; consequently, it remains open if the applied analytical methods are indeed suitable for their use.
- Furthermore, a quite high number of other concerns add additional uncertainty on the performed biosimilarity exercise.

On the top of that, although not directly related to the biosimilarity part, a number of serious concerns have been identified which leave a quite high level of uncertainty on the overall quality development of the biosimilar development open. A poor-quality dossier which is partly far away from what is expected for an MAA in Europe includes for many sections only high-level overviews and summaries whereas core information and essential details required for an assessment are missing. Furthermore, serious discrepancies and non-alignment of the information presented in the different sections are noticed. Finally, only selected and partial results were presented without legends, explanation or conclusions, and not discussed. Consequently, these issues resulted in several MOs addressing:

- The insufficient description of the drug substance manufacturing process and its control.
- A major safety risk by using undocumented materials of animal origin as raw material/cell culture component and for affinity chromatographic purification of the drug substance.

- Poor level of information on the development of a control strategy for drug substance and drug product manufacturing process which hamper an assessment and conclusion on the appropriateness of the control strategy.
- Inadequate or missing process validation of the substance and drug product manufacturing process; thus, the validity of the manufacturing process is not demonstrated.
- Missing of essential information required to understand CQAs of the drug substance in combination with the manufacturing process, including but not restricted to its structure, its post-translational modifications, its variants, its degradation products, its impurity profile and its biological activity.
- Essential core information to enable an evaluation of the respective analytical portfolio to control DS and DP critical quality attributes was not provided. Thus, assessment of their suitability was not possible.
- Information on reference standard and its provenience was incomplete. Furthermore, proposed analytical qualification is incomplete and the reference standard seems to be unstable.
- Viral safety: Essential core information on the design, conduct and outcome of the virus validation studies has not been provided; thus making an assessment of these validation studies not possible.
- The manufacturing process of the solvent WFI was poorly described. In addition, any information on process validation of the WFI manufacturing process is completely missing. As these issues add to the uncertainty regarding the safety profile of the reconstituted product, a major objection was raised.
- The missing nitrosamine risk assessment leaving additional uncertainties regarding the safety profile of drug product open.

Clinical

Pharmacodynamics

The evaluation of bioequivalence in the primary PD endpoints was inappropriately based on the 90% confidence intervals instead of 95%. Furthermore, the equivalence margins were widened without proper justification. The design is inadequate to properly estimate the coefficient of variation in the respective endpoints.

For the primary PD endpoints TGA ETP (AUC), TGA Lag Time (AUC) and TGA Peak height (AUC), the 90% CI of the ratios of AryoSeven/NovoSeven of both doses tested (90 µg/kg and 270 µg/kg) are completely contained within the bioequivalence interval of (0.8–1.25). However, the resulting 90% CIs for TGA ETP and TGA peak heights not covering "1" indicate a statistically significant difference in these parameters.

The point estimate for AUC D-Dimer was 0.9813 (90% CI: 0.7371 to 1.3064) for the lower dose of 90 µg/kg and the point estimate for the higher dose of 270 µg/kg was 0.8416 (90% CI: 0.633 to 1.1189). The results of the AUC comparison showed that the 90% CIs were outside of the equivalence margin of (0.80 to 1.25) for both concentrations.

The applicant has widened the equivalence margins for the peak value of F1.2 and for the AUC of the lower dose (90 µg/kg) without adequate justification. For the higher dose of 270 µg/kg, the standard margin of 0.8 to 1.25 was used and the point estimate was 1.0657 (90% CI: 0.6937 to 1.6372), where the 90% CI were outside of the proposed margin of (0.80 to 1.25).

The PD similarity claim between AryoSeven and NovoSeven is not supported. This is considered of a major concern as this part of the biosimilarity exercise is intended to substitute relevant clinical comparative data. The Study UGA 2014-01 contains major deficiencies in relation to:

- the coverage probability of confidence intervals used for PD similarity assessment (90% used instead of desired 95%)
- the inadequacy of the choice of equivalence margins to evaluate PD equivalence (no clinical justification for using 0.8-1-25)
- the widening of the acceptance interval for F1.2 AUC (90 µg/kg) and F1.2 peak (both dose levels). In the regulatory context, while the scaled-average-bioequivalence approach could be used for highly variable drugs in the framework of PK, this cannot be extended to PD endpoints. Moreover, even in that framework, it would require cross-over data from semi or replicated design (for proper estimation of intra-subject variability using the reference product), which was not the case with the study at hand. Finally, widening would only be applied for peak (not AUC) and limited to a maximum of [69.84 – 143.19%] which is exceeded (90%CI F1.2 peak = [0.6524 1.5327])
- high variability emerged from the entire co-primary PD parameter set, and similar results could only be obtained for 2 endpoints (TGA lag time and D-dimer peak) out of 7. Indeed, the resulting 90% CIs for TGA ETP and TGA peak heights do not cover "1" indicating a statistically significant difference and the 90% CIs for D-Dimer AUC, F1.2 AUC and F1.2 peak were outside of the equivalence margin for both dose levels.

For a robust interpretation in an equivalence setting, the results of the PD comparisons should lead to similar conclusions, taking into account an adequate equivalence margin chosen for statistical and clinical reasons, which was not provided. Based on the grounds presented, the study has formally failed to show equivalence in PD between HemAryo/AryoSeven and NovoSeven. The applicant is asked

a) to comment in which way those methodological concerns can be overcome to allow for a sound similarity evaluation of the predefined primary PD parameters

b) to discuss why a lack of PD comparability should not be considered clinically relevant with regard to the chosen endpoints and the different doses used.

Furthermore, no results were presented for the additional (secondary) endpoints.

Efficacy

The analysis of bleeding rates is currently limited because some patients were still in the early stages of follow-up when the database of pivotal study UGA 2014-01 was frozen for statistical analysis.

Although almost all bleeding resolved completely within 12 hours in the two pivotal trials, no direct comparison has been made with historical NovoSeven data.

Safety

Study UGA 2014-01 is still ongoing and safety (and immunogenicity) data should be submitted for the remaining 6 patients who are still in follow-up.

In the sole studies with a comparative safety evaluation, AEs were more frequently reported in the HemAryo group than in NovoSeven arm:

IRTC201104266302N2 (haemophilia A/B with inhibitors): AEs in 3/31 (9.7%) patients receiving HemAryo (nausea, rash and headache), and in 1/35 patient (2.9%) in the NovoSeven arm (headache).

IRTC201104266302N1 (congenital FVII deficiency): A total of 16 AEs have been reported in 8 patients (22.9%) of the AryoSeven arm (12 AEs) and in 3 patients (9.7%) of the NovoSeven arm (4 AE).

According to the applicant, none of these potentially evocative signs of infusion-related reactions were evaluated by the investigators as possibly or probably related to study medications. Nevertheless, due to the several deficiencies of these studies, the relevance and the usefulness of safety data from these studies are doubtful.

Immunogenicity

Immunogenicity of the API was not properly assessed in the pivotal clinical studies. First, the proposed assay is irrelevant for the assessment of the immunogenicity of the API. It is unspecific and recognises **all** kind of inhibitors raised against mostly **all** compounds of the human blood coagulation cascade, and even does not comprise the API.

According to the inclusion and exclusion criteria of studies UGA 2014-01 and 2014-02, previous treatment with AryoSeven or NovoSeven was allowed whereas subjects with pre-existing antibodies against FVII were to be excluded. This renders the immunogenicity assessment of patients included in the PK and PK/PD groups of the pivotal studies futile.

It has been concluded during the scientific advice procedures for HemAryo that immunogenicity would be assessed in FVII deficient patients with no or only few previous FVIIa treatments. For this purpose, it was planned to enrol a separate patient group in study UGA 2014-02. However, this part of the study was not carried out.

Despite the favourable immunogenicity results obtained in the post marketing study, the comparability of patient populations (especially with regard to previous exposure to rFVIIa) and the assays used to detect antibodies against FVII (especially the quality with which the analyses were performed) still needs to be discussed in order to relate these results to literature data.

Overall, the applicant should provide an integrated immunogenicity assessment taking the deficiencies in the current evaluation into account and elaborate on plans to potentially evaluate immunogenicity of HemAryo in future studies.

5.4. Discussion on biosimilarity

In conclusion, the points raised in the quality Major Objection addressing the applicant's biosimilarity exercise together with the relatively high number of additional other concerns leave doubts on the applicant's principal understanding of principles of GMP and conducted biosimilarity approach. On top of missing confirmation of biosimilarity, a number of other Major Objections addressing various sections of Module 3 have been raised which question the applicant's whole quality development programme. From the CHMP's understanding the raised issues in the Major Objection cannot be easily solved. Thus, the obviously best way to address the serious issues would be to, first of all, get an in-depth understanding on API's CQAs, and second to get insight into process variability based on a thoroughly controlled, state of the art USP and DSP in absence of critical materials of animal origin, and third, to repeat the biosimilarity exercise based on a sufficient number of biosimilar and RMP batches, taking into account several points addressed in the Major Objections. Taken together the current content and structure of Module 3 is far away from quality standards expected for an MAA in Europe.

Pharmacokinetic similarity of HemAryo to EU-NovoSeven was demonstrated in two pivotal Phase III PK/PD studies, based on primary PK parameters. The secondary endpoint analysis supports the

primary results. Unless the requested information on the analysis of one patient in question is contradictory, biosimilarity can be inferred at the PK level.

The equivalence margins for PD assessment were widened without proper justification. High variability in the chosen endpoints should be handled by adjusting the sample size, not lowering the acceptance criteria. Furthermore, the underlying design is flawed form a methodological perspective to adequately estimate the coefficient of variation in the respective endpoints. In addition, the 90% confidence intervals were used as the basis for the assessment of bioequivalence. In the PD endpoints, the 95% confidence intervals would be required to establish bioequivalence.

Significant variability emerged from the co-primary PD parameter set chosen, where most of the co-primary PD parameters did not meet the equivalence margins. Thus, the study has formally failed to show equivalence in PD between HemAryo/AryoSeven and NovoSeven; this is raised as a Major Objection. In addition, results of the secondary endpoints were apparently assessed but not provided.

As efficacy was assessed in the immunogenicity follow-up period without a comparator, the comparison of haemophilia A/B patient bleeding rates with historical data from the NovoSeven trials should be performed and discussed. Similarity on the efficacy level can only be concluded, provided that the concerns raised are sufficiently answered.

No specific safety concerns were identified from the analysis of AEs across all studies conducted and the observed adverse events reflect the safety profile of the reference product NovoSeven.

Comparative immunogenicity was not assessed in the pivotal clinical trials, since the reference product was not included in the immunogenicity follow-up period. Post marketing immunogenicity data need to be discussed in terms of validity and comparability to external literature data and an overall integrated immunogenicity assessment needs to be provided.

5.5. Extrapolation of safety and efficacy

The applicant provided PK, PD and efficacy data for HemAryo in patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX. PK and efficacy data were also established in patients with congenital FVII deficiency. A marketing authorisation for all indications of the reference product NovoSeven is applied for, including treatment of patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration, patients with acquired haemophilia and patients with Glanzmann's thromboasthenia. Extrapolation to the indications not covered by the pivotal clinical studies is in principle possible provided that comparability of HemAryo to NovoSeven has been convincingly demonstrated and that the respective issues at the quality, non-clinical and clinical levels have been resolved. However, such extrapolation cannot be granted unless a thorough justification based on a scientific discussion of the mechanism of action of HemAryo in the different indications has been provided.

5.6. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, HemAryo is considered not biosimilar to Novoseven. Therefore, a benefit/risk balance comparable to the reference product cannot be concluded.