22 May 2014
EMA/CHMP/279301/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Nuwiq

International non-proprietary name: SIMOCTOCOG ALFA

Procedure No. EMEA/H/C/002813/0000

Note

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

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List of abbreviations

AE	Adverse event
APC	Activated protein C
APTT	Activated partial thromboplastin time
AUC	Area under the curve (from baseline to infinity)
AUC_{norm}	Area under the curve normalised to the dose administered
BDD	B-domain-deleted
BE	Bleeding episode
BHK	Baby hamster kidney
BMI	Body mass index
BW	Body weight
BU	Bethesda Unit
CBT	Cuticle bleeding time
Cl	Cell-line
CD4	Cluster of differentiation 4
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese hamster ovary
CHR	Chromogenic
CL	Clearance
C_{max}	Maximum plasma concentration
$C_{maxnorm}$	Maximum plasma concentration normalised to the dose administered
Da	Daltons
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ED	Exposure day
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FVIII	Blood coagulation factor VIII
FVIII:C	FVIII coagulant activity
FIX	Blood coagulation factor IX
FIXa	Blood coagulation factor IX activated
FX	Blood coagulation factor X
FXa	Blood coagulation factor X activated
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HJHS	Haemophilia Joint Health Score
Human-cl rhFVIII	Human cell line recombinant human factor VIII
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee

IMP	Investigational medicinal product
IPC	In-process control
ITT	Intention-to-treat
IU	International units
IV	Intravenous
IVR	*In vivo* recovery
KLH	Keyhole limpet haemocyanin
MAA	Marketing authorisation application
MCB	Master cell bank
MHC	Major histocompatibility complex
MAH	Marketing authorisation holder
MRT	Mean residence time
Neu5Ac	*N*-acetylneuraminic acid
Neu5Gc	*N*-glycolylneuraminic acid
OS	One-stage
PBMC	Peripheral blood mononuclear cells
PD	Pharmacodynamic
pdFVIII	plasma-derived human FVIII
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic
PP	Per-protocol
PRAC	Pharmacovigilance Risk Assessment Committee
PTM	Post-translational modification
PT	Preferred term
PTP	Previously treated patient
PUP	Previously untreated patient
rDNA	Recombinant deoxyribonucleic acid
rFVIII	Recombinant FVIII
RMP	Risk management plan
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
S/D	Solvent/detergent
SmPC	Summary of Product Characteristics
$T_{1/2}$	Half-life
TSE	Transmissible spongiform encephalitis
T_{max}	Time to reach maximum plasma concentration
USP	United States Pharmacopoeia
V_{ss}	Volume of distribution at steady state
VWF	Von Willebrand factor
WCB	Working cell bank
WBCT	Whole blood clotting time
WFI	Water for injections

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Octapharma AB submitted on 29 May 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Nuwiq, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

"Treatment and prophylaxis of bleeding (also during and after surgery) in patients with haemophilia A (congenital factor VIII deficiency).
Nuwiq is also indicated in haemophilia A patients with known allergic reactions to mouse or hamster protein, in which hamster cell derived rFVIII are contraindicated.
Nuwiq is appropriate for use in adults and children of all ages, including newborns."

The applicant changed the application during the evaluation to *"Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). Nuwiq can be used for all age groups."*

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that simoctocog alfa was considered to be a new active substance.

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

Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0214/2012 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-001024-PIP01-10-M01 was not yet completed as some measures were deferred. The PDCO discussed the completed studies on EMEA-C1-001024-PIP01-10-M01 and considered that these are compliant with the latest Agency's Decision (P/0214/2012) of 28 September 2013.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible

similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request(s) for consideration

New active substance status

The applicant requested the active substance simoctocog alfa contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Scientific Advice

The applicant did not seek scientific advice from the Committee for Medicinal Products for Human Use (CHMP).

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer(s) of the active substance

Octapharma AB
Elersvägen 40
Stockholm
11275 Sweden

Manufacturer responsible for batch release

Octapharma AB
Elersvägen 40
Stockholm
11275 Sweden

1.3. Steps taken for the assessment of the product

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

Rapporteur: Jan Mueller-Berghaus Co-Rapporteur: Andrea Laslop

- The application was received by the EMA on 29 May 2013.
- The procedure started on 26 June 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 16 September 2013. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 13 September 2013
- During the meeting on 10 October 2013 the Pharmacovigilance Risk Assessment Committee (PRAC) adopted the PRAC Rapporteur RMP Assessment Report.
- During the meeting on 24 October 2013, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24 October 2013.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 17 January 2014.
- The Integrated Inspection Report and related appendices of the inspections carried out at the two clinical investigator sites was issued on 28 February 2014.
- The report of the inspection carried out at the manufacturing site of the active substance was issued on 28 February 2014.
- During the meeting on 6 March 2014, the PRAC adopted a PRAC RMP Advice and assessment overview.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 13 March 2014.
- During the CHMP meeting on 20 March 2014, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 15 April 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Outstanding Issues to all CHMP members on 30 April 2014
- During the meeting on 8 May 2014, the PRAC adopted the PRAC Rapporteur RMP Assessment Report.
- During the meeting on 22 May 2014, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Nuwiq.

2. Scientific discussion

2.1. Introduction

Haemophilia A is an inherited sex-linked disorder of blood coagulation in which affected males do not produce functional coagulation factor VIII (FVIII) in sufficient quantities to achieve satisfactory haemostasis. The incidence of congenital haemophilia A is approximately 1 in 10,000 births. Disease severity is classified according to the level of FVIII activity (% of normal) as mild (>5% to <40%), moderate (1% to 5%) or severe (<1%). This deficiency in FVIII predisposes patients with haemophilia A to recurrent bleeding episodes (BEs) in joints, muscles or internal organs, either spontaneously or as a result of accidental or surgical trauma.

Without adequate treatment, these repeated haemarthroses and haematomas lead to long-term sequelae with severe disability. Other less frequent, but more severe bleeding sites, are the central nervous system, the urinary or gastrointestinal tract, eyes and the retro-peritoneum. Patients with haemophilia A are at high risk of developing major and life-threatening BEs after surgical procedures, even after minor procedures such as tooth extraction.

The development of cryoprecipitate and subsequently FVIII concentrates, obtained by fractionation of human plasma, provided replacement FVIII and greatly improved clinical management and life expectancy of patients with haemophilia A. Replacement therapy with exogenous FVIII successfully adjusts haemostasis in these patients, temporarily. Prophylaxis with FVIII concentrates is currently the preferred treatment regimen for patients with severe haemophilia A, especially in very young patients. The majority of patients receiving prophylaxis are treated 3-times weekly or every other day at a dose of 25–40 international units (IU)/kg (or 15–25 IU/kg in an intermediate dose regimen), although an escalating dose regimen is also used. However, on-demand treatment is still the predominant replacement approach in many countries.

The most serious complication in the treatment of haemophilia A is the development of neutralising antibodies (inhibitors) against FVIII, rendering the patient resistant to replacement therapy and thereby increasing the risk of unmanageable BEs, particularly arthropathy, and disability.

Nuwiq is a recombinant B-domain-deleted (BDD) rFVIII human FVIII concentrate that is produced in genetically modified human embryonic kidney (HEK) 293F cells. The rationale for using a human cell line for rFVIII expression was in order to more closely mimic the pattern of post-translational modifications (PTMs) of endogenous FVIII, resulting in elimination of potentially antigenic epitopes created during production in non-human cells. *N*-glycosylation of Nuwiq shows the same distribution of *N*-glycosylation types outside the FVIII B-domain as human plasma-derived FVIII (pdFVIII). The only type of sialic acid present is *N*-acetylneuraminic acid (Neu5Ac). The sialic acid *N*-glycolylneuraminic acid (Neu5Gc), reported to be antigenic in man, was not detected.

Nuwiq is presented as lyophilised powder and is reconstituted with 2.5 mL of sterilised water for injections in a syringe in single-dose vials containing 250 IU, 500 IU, 1000 IU, and 2000 IU of recombinant factor VIII per vial.

2.2. Quality aspects

2.2.1. Introduction

Nuwiq is intended for treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). The active substance of Nuwiq, simoctocog alfa is a B-domain deleted (BDD) rFVIII produced in genetically modified human embryonic kidney (HEK) 293F cells. The harvested product is concentrated and purified by a series of chromatography steps. No animal proteins are used in the purification process and no human albumin is used as a stabiliser in the manufacture of *Human-cl rhFVIII*.

2.2.2. Active substance

Human-cl rhFVIII is a glycoprotein consisting of 1440 amino acids with an approximate molecular mass of 170 kDa, comprising the FVIII domains A1-A2 + A3-C1-C2 whereas the B-domain, present in the full-length plasma-derived FVIII, has been deleted and replaced by a 16 amino acid linker. The linker sequence was inserted in the 170 kDa single-chain molecule of *Human-cl rhFVIII* between the A2 and A3 domain. The first eight amino acids of the linker sequence correspond to the first eight N-terminal amino acids of the B-domain, while the remainder arginine-rich octapeptide was chosen to provide a recognition site for furin or furin-like proteases in order to ensure similar proteolytic processing as for the full-length FVIII molecule.

Proteolytic cleavage of the *Human-cl rhFVIII* 170 kDa single chain results predominantly in the fully active light-chain/heavy-chain heterodimer. Once activated by thrombin cleavage, the resulting rFVIIIa has the same structure as endogenous FVIIIa.

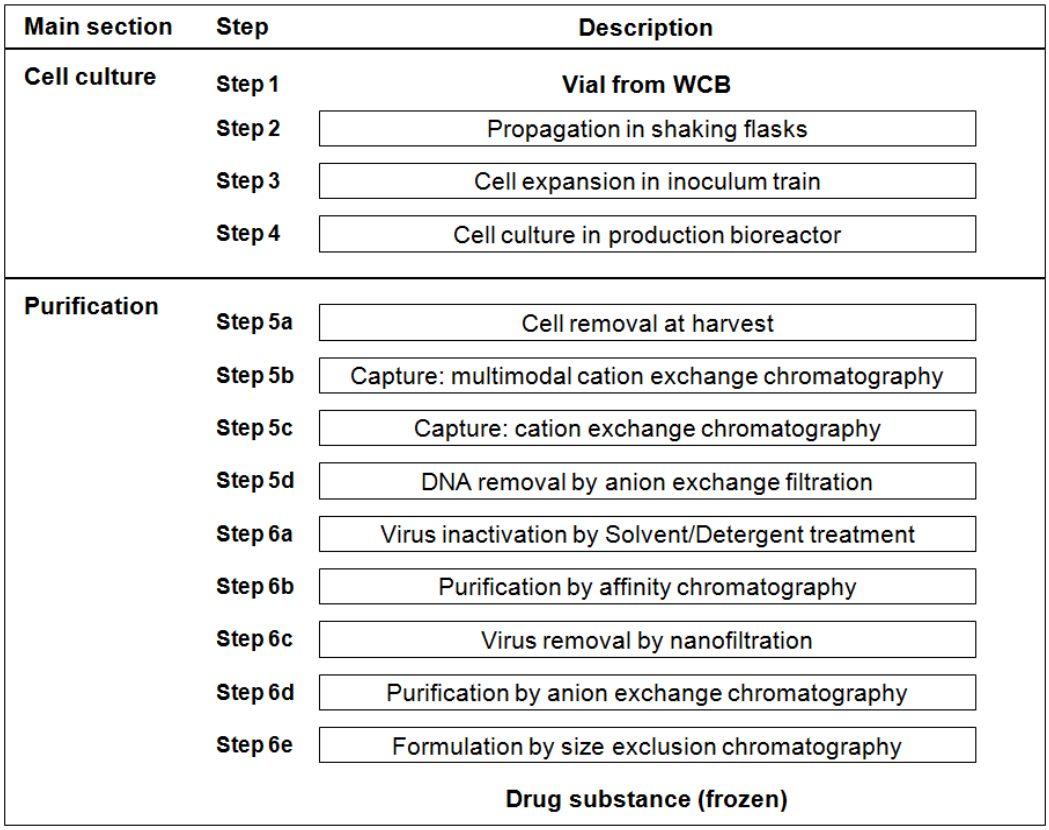
The characteristics of the post-translational modifications (PTMs) are in conformity with those of human plasma-derived FVIII. Another characteristic of *Human-cl rhFVIII* is the entire sulphation at tyrosine 1680 as found in pdFVIII. Sulphation at tyrosine 1680 is of importance for binding to von Willebrand factor (VWF).

Thus, compared with the full-length FVIII, critical structural features for the coagulant function are retained in *Human-cl rhFVIII*.

Manufacture

The manufacturing process is divided into an upstream cell cultivation process and a downstream purification process (Figure 1).

Figure 1: Flow chart of Nuwiq active substance manufacturing process



Control of materials

Cell banking system, characterisation and testing

A two-tiered cell bank system was established. The manufacture of the master cell bank (MCB) and working cell bank (WCB) is sufficiently detailed and generally in compliance with International Conference on Harmonisation (ICH) guidelines ICH Q5B and ICH Q5D. The provided information on the origin, source, and history of the cell substrate is generally acceptable.

The expression cell line 293F is derived from HEK 293F cells. The host cell line HEK 293F and all its generated derivatives have exclusively been cultured in serum-free expression medium, free of any materials of animal or human origin.

Tests for sterility and for the presence of mycoplasmas were conducted on both the MCB and WCB following the recommendations as outlined in ICH Q5D. Sterility and absence of mycoplasmas was confirmed. Additionally, sterility and mycoplasma tests are performed according to the European Pharmacopoeia (Ph. Eur.) on the bulk harvest from each WCB vial used for production.

Overall, the applicant, in line with ICH Q5B, has described the origin and modifications of the host cell system, the vectors, the rFVIII gene construct and the final gene construct in the

recombinant cell line in sufficient detail. The rationale for the cloning strategy, the selection of vectors and the final recombinant clone to establish the cell line appear justified.

In conclusion, productivity, viability and genetic stability, as well as the absence of adventitious agents and mycoplasma is in principle confirmed for all cell banks for the intended *in vitro* cell age.

Process validation and/or evaluation

Process validation covered one production campaign from revival of a frozen WCB vial to purified active substance and one production campaign starting with a second WCB vial.

A major objection was raised during the evaluation regarding the validation of the manufacturing process, however this has been sufficiently addressed and considered resolved.

Characterisation

In line with ICH Q6B the applicant has performed an extensive characterisation study on intermediates, active substance and finished product from validation batches of the non-clinical, clinical and commercial process.

Simoctocog alfa (human coagulation factor VIII (rDNA)) is a purified protein of 1440 amino acids. The amino acid sequence is comparable to the 90 + 80 kDa form of human plasma factor VIII (i.e. B-domain deleted).

The structural analysis and physicochemical characterisation confirmed the expected properties for a rFVIII product. Analysis of the product indicates a fully sulphated glycoprotein with an oligosaccharide and sialic acid composition comparable to human pdFVIII.

Tyrosine sulphation of human *cl-rFVIII* was found comparable to pdFVIII with six potential sulphation sites fully sulphated. It is known that these sites are important for FVIII activity and on the ability of FVIII to interact with VWF.

The data provided on the biological *in vitro* characterisation provides the justification that Nuwiq is converted to the same activated FVIII as native pdFVIII. Characterisation data of biological activity was presented for the interaction with VWF, assessment of the co-factor activity, as well as for the activation and inactivation rates.

Product- and process-related impurities have been identified and are well under control. The impurity profile is comparable and consistent in different batches.

Overall, the applicant has provided sufficiently detailed data for the characterisation of the active substance, including structure and general properties, as well as documents indicating that the quality of the active substance is well controlled.

Comparability exercise for the active substance

The manufacturing process development, starting from the non-clinical (P1) to the early clinical (P2a) and subsequently to late clinical and intended commercial manufacturing process (P2b), has been sufficiently described. Comparability of batches derived from the different processes has been demonstrated during the characterisation work.

Specification

In principle, the specifications, which have been set following regulatory requirements or based on batch history, are considered acceptable.

The specifications for the active substance mainly comprise the following parameters: appearance, identity, pH, polypeptide chain composition, molecular size distribution, total protein, specific activity, *N*-glycan fingerprinting, bio-burden, bacterial endotoxins, potency and purity. The biological activity of rFVIII was characterised by an adequate set of methods.

FVIII coagulant activity (FVIII:C) is quantified using an assay based on a chromogenic (CHR) substrate, which measures the FVIII cofactor effect on the amount of FXa generated in a mixture containing phospholipids, calcium ions, and excess amounts of FIXa and FX.

Human-cl rhFVIII was shown to have a similar biological functionality compared to pdFVIII.

Stability

The primary and supportive studies were performed according to the current ICH guidelines. Based on the data provided the proposed shelf-life for the active substance is acceptable.

2.2.3. Finished Medicinal Product

The Nuwiq finished product is a white sterile lyophilised powder and solvent for solution for injection. The lyophilised powder is supplied in single-dose vials containing 250 IU, 500 IU, 1000 IU, and 2000 IU of rFVIII per vial. The finished product is formulated with sodium chloride, sucrose, calcium chloride dihydrate, arginine hydrochloride, sodium citrate dihydrate, poloxamer 188. Before use, the lyophilised powder is reconstituted with a single-dose solvent pre-filled syringe containing 2.5 mL of water for injections (WFI).

The reconstituted solution is a clear, colourless solution, practically free from visible particles, containing 100 IU / 200 IU / 400 IU / 800 IU FVIII:C/mL. The concentration of each of the excipients is the same for all strengths, only the recombinant FVIII concentration varies. All excipients are of Ph. Eur. quality.

The package for the final product is a combination package containing a vial with the lyophilised active substance, the solvent WFI and the medical devices necessary for correct use. The devices include a vial adaptor, a butterfly needle and alcohol swabs. All devices are CE-certified.

Pharmaceutical Development

The pharmaceutical development has been sufficiently described. Changes made from initial non-clinical to early and late clinical batches and to process validation batches of commercial scale are explained and have been justified. The information provided on the development studies conducted to establish the dosage form, the formulation, manufacturing process, container closure system, microbiological attributes and compatibility is appropriate for the purpose specified in the application.

No significant changes are made in the manufacturing process between the clinical lots and the lots intended for marketing.

Manufacture of the product

The manufacturing process consists of thawing and pooling of active substance, dilution to the final formulated solution, sterile filtration, filling, freeze-drying, capping, integrity test, visual inspection, labelling and packaging.

A comprehensive description of the manufacturing process including appropriate in-process controls (IPCs) has been provided. Critical steps have been identified and are appropriately controlled. Process validation has been performed with eight batches of the finished product covering all four intended commercial strengths. In general, the provided process validation results demonstrated that the Nuwiq finished product can be consistently manufactured with the intended commercial manufacturing process.

A batch formula with all ingredients and their amounts on a per batch basis as well as a reference to their quality standards is given.

All excipients used for the formulation of the final Nuwiq finished product are in compliance with the current edition of the Ph. Eur. specifications, analytical procedures and their validations. Justification of the specifications comply with the relevant monographs of the Ph. Eur.

Product specification

The specifications are in line with the Ph. Eur. monograph for FVIII (rDNA). For non-compendial specifications the limits are based on experience from clinical and/or process validation lots, and the established acceptance limits can in principle be accepted. The batch analyses data demonstrate consistency in manufacturing and that a product of consistent quality and composition is obtained.

Container closure system

The container closure system for the Nuwiq finished product consists of vials Ph. Eur. type I glass closed with bromobutyl lyophilisation stoppers. The vials are sealed with aluminium flip off caps, which have no immediate contact with the finished product.

The container closure system is satisfactorily described and complies with Ph. Eur. requirements. Medical devices that are part of the finished product are also described in appropriate detail.

Stability

Stability studies in accordance with ICH Q5C, in real time and under accelerated and excursion conditions, were performed. All studied parameters remained within the specification limits during the stability studies.

Photo-stability studies indicate a slight loss in FVIII potency and specific activity, when stored under light, requiring storage of the vials protected from light. A respective statement is included in the product information.

The stability programme is considered satisfactory. The results generated during the stability studies support the proposed shelf life and storage conditions as defined in the Summary of Product Characteristics (SmPC).

Adventitious agents

TSE compliance

The active substance of Nuwiq is produced in genetically engineered HEK 293F cells using serum-free medium. The MCB and WBC that have been established for the expression of Nuwiq are free from transmissible spongiform encephalitis (TSE) risk substances. No material of animal origin is added during production of Nuwiq.

Virus safety

Nuwiq is expressed using genetically engineered HEK 293F cells which are of human origin. The fermentation process for Nuwiq is in serum-free medium. No other material of animal or human origin is added during production of Nuwiq. This minimises possible contamination by adventitious viruses. The cells used for production of Nuwiq have been screened for viruses. The choice of viruses and testing programme is considered sufficient. No evidence of viral contamination was detected in the MCB, WCB or cells at the limit of *in vitro* cell age used for the expression of Nuwiq.

The purification process of Nuwiq includes several orthogonal steps for inactivation/removal of enveloped viruses. Solvent/detergent (S/D) treatment has been shown to be effective for inactivation of a panel of model enveloped viruses, even under robustness conditions. Further removal of enveloped viruses takes place during the virus retentive filtration step which has been also evaluated for the reduction of non-enveloped viruses such as parvoviruses. Robustness of the virus retentive filtration step has been demonstrated with effective removal of the model porcine parvovirus even under conditions of increased pressure and filtration volume. In addition, multiple chromatography steps are performed and are expected to further contribute to the virus reduction capacity of the Nuwiq manufacturing process.

In summary, the virus and TSE safety of Nuwiq has been sufficiently demonstrated. Nuwiq is considered safe with respect to a potential transmission of TSE and viruses.

Finished Product – Solvent – Water for injections

The WFI for reconstitution of freeze-dried FVIII is delivered as a pre-filled syringe consisting of a siliconised glass barrel, a siliconised plunger, and a closure system composed of a tip cap with a Luer lock and a tamper-evident seal.

The manufacturing process, IPCs, as well as control of critical steps have been adequately described. Furthermore, a sufficiently detailed process validation, including a justification for the used bracketing approach, has been provided by the applicant.

The WFI pre-filled syringes are manufactured and tested for compliance with the appropriate specifications. The methods and controls used for the manufacturing, packaging and storage of the WFI pre-filled syringes are in accordance with current Good Manufacturing Practices.

The information provided in the dossier for the solvent is considered sufficient to conclude that the WFI in a syringe meets Ph. Eur. requirements for sterile WFI and is manufactured under GMP compliant conditions in a fully validated process.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Nuwiq is a B-domain-truncated rFVIII, manufactured in HEK 293F without the use of any human- or animal-derived auxiliary material (except the cell line). The generation of the B-domain-truncated rFVIII gene construct, the characterisation of the host cell line and cloning of the rFVIII transgene into the host cell line have been performed in compliance with ICH Q5B and Q5D. The cell banking strategy with a two-tiered banking system and an end-of-production cell line is in line with ICH Q5D.

A major objection was raised during evaluation concerning the validation of the manufacturing process. This has been sufficiently addressed and is considered resolved. Data from pre-validation and validation conformance lots have been provided and indicated that the cell culture process and the downstream purification process operate within pre-defined operating ranges, leading to an active substance of consistent quality.

Two steps of the Nuwiq manufacturing process have been evaluated for their virus removal/inactivation capacity: the S/D treatment step and the retentive virus filtration step. In addition, multiple chromatography steps are performed, which are expected to further contribute to the virus reduction capacity of the Nuwiq manufacturing process. The virus and TSE safety of Nuwiq has been sufficiently demonstrated. Nuwiq is considered safe with respect to a potential transmission of TSE and viruses.

No quality aspects impacting the benefit-risk balance were identified for Nuwiq.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Overall, the applicant has provided sufficiently detailed data and documents indicating that the quality of the active substance and finished product are well controlled.

Information about the manufacturing process, the validation of this process and the characterisation and quality control of the active substance is of acceptable quality.

The manufacturing process of the finished product is described in sufficient detail and has been satisfactorily validated. Risk analyses to categorise critical quality attributes and process parameters are provided. Specification limits and analytical methods are suitable to control the quality of the active substance and finished product.

The stability programme is in general considered satisfactory. The results generated during the stability studies support the proposed shelf life and storage conditions as defined in the SmPC.

2.2.6. Recommendations for future quality development

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

2.3. Non-clinical aspects

2.3.1. Introduction

The biological activity of *Human-cl rhFVIII* was analysed *in vitro*. *In vivo*, non-clinical pharmacology and haemostatic properties have been evaluated in a haemophilia A dog study. Non-clinical pharmacology was also evaluated in Cynomolgus monkeys during a preliminary repeated-dose toxicity study. Toxicology studies were performed in rats and monkeys and a local tolerance study was performed in rabbits.

2.3.2. Pharmacology

Non-clinical pharmacology of *Human-cl rhFVIII* was investigated in different *in vitro* studies and in animals models (Table 1).

Table 1: Summary of pharmacology studies

Type of study	Species/ Test system	Administration	Study number
Primary PD and safety pharmacology	Biological activity (different assays)	*In vitro*	230CBA140/00 230CBA139/01
	Dog	IV injection	Oct. 10, 2007 OC11-0200
	Monkey	IV injection	DWL0001/063743
	Monkey	IV injection	DWL0002/064067

IV = Intravenous; PD = pharmacodynamic.

In vitro, the investigated parameters included FVIII:C, determination of FVIII antigen, activation and inactivation with human α-thrombin, thrombin generation, FXa generation, inactivation by activated protein C (APC) and binding to VWF.

The pharmacodynamic (PD) activity of *Human-cl rhFVIII* was evaluated in dogs by assessment of haemostatic efficacy, FVIII levels, cuticle bleeding time (CBT), activated partial thromboplastin time (APTT) and whole blood clotting time (WBCT). FVIII levels and APTT were also assessed as part of two repeat-dose toxicology studies in Cynomolgus monkeys.

PK properties were also evaluated in the haemophilia A dog study and on Day 1 of a repeated-dose toxicity study in Cynomolgus monkeys.

Primary pharmacodynamic studies

In vitro studies

The biological activity of *Human-cl rhFVIII* was tested *in vitro* in comparison to a pdFVIII and currently marketed rFVIII products (two full length and one BDD rFVIII). The testing comprised several *in vitro* studies of the function of *Human-cl rhFVIII*.

Results obtained from non-clinical and process validation batches for the ratio of OS clotting assay over CHR assay for *Human-cl rhFVIII* were in the range of that obtained for one of the full-length rFVIII and higher than those of the BDD rFVIII and the other full-length rFVIII. It could be concluded that *Human-cl rhFVIII* batches manufactured with the intended clinical manufacturing process and non-clinical batches produced upon validation of the manufacturing process exhibit satisfactory FVIII:C as measured by the CHR and OS assays.

The measurement of FVIII:C/mg protein gives information about the quality of function and purity of the product. FVIII:C was measured by a CHR assay and protein was determined by amino acid analysis. The results indicated that *Human-cl rhFVIII* batches have a comparatively high specific FVIII:C, with the results being between 9,400 and 10,800 IU FVIII:C/mg protein.

FVIII antigen was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) based on the use of two commercial monoclonal antibodies directed against the 80 kDa protein chain. The results indicated that all FVIII molecules, present in *Human-cl rhFVIII*, have FVIII activity. The ratio between FVIII activity and FVIII antigen in *Human-cl rhFVIII* is close to one, which is the ideal situation, indicating that the FVIII protein is intact and fully active.

The ability of *Human-cl rhFVIII* to generate thrombin at a normal rate and at physiological levels following triggering of coagulation was demonstrated by the Thrombogram-Thrombinoscope assay. It could be concluded that *Human-cl rhFVIII* exhibits full ability to generate sufficient and physiological amounts of thrombin when needed and that the generation of thrombin occurs with a satisfactory rate. The consistency between batches was very good and there were no differences between drug substances and drug products.

The ability of *Human-cl rhFVIII* to participate as a cofactor in the formation of factor Xa was found to be similar to rFVIII and pdFVIII products.

The inactivation pattern of *Human-cl rhFVIII* by APC according to the OS assay showed an expected interaction for a FVIII protein.

The kinetics in binding and affinity of *Human-cl rhFVIII* to human VWF was analysed using surface plasmon resonance with Biacore instruments. The affinity of *Human-cl rhFVIII* for VWF was significantly higher than for two full-length rFVIII comparators and similar to a BDD rFVIII comparator. Furthermore, in comparison with other rFVIII products, a higher portion of the FVIII protein present in *Human-cl rhFVIII* was able to bind to human VWF.

Primary pharmacodynamics in dogs

A study was performed to evaluate the haemostatic efficacy of *Human-cl rhFVIII* in the canine model of haemophilia A using the CBT assay, and by testing the clotting function using the WBCT and APTT tests. FVIII:C was monitored by CHR and OS assays. Two dogs were treated with *Human-cl rhFVIII* or a marketed BDD rFVIII in a cross-over design. Both rFVIII concentrates were administered by intravenous (IV) injection at a dose of 125 IU FVIII:C/kg within 1 hour of reconstitution. Haemostatic efficacy and PD activity were found to be similar.

Primary pharmacodynamics in Cynomolgus monkeys

FVIII:C and APTT were also assessed as PD parameters of *Human-cl rhFVIII* in the course of the repeated-dose toxicity studies (DWL0001/063743 and DWL0002/064067). With repeated doses in monkeys, there were decreased levels of endogenous FVIII activity and evidence of inhibitors against *Human-cl rhFVIII* and endogenous FVIII due to the generation of FVIII neutralising antibodies. The antibody response was due to repeated injections of species different recombinant human factor VIII

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies have been submitted.

Safety pharmacology programme

Safety pharmacology of *Human-cl rhFVIII* was assessed as part of the primary PD study in haemophiliac dogs and a 28-day repeated-dose toxicity study in Cynomolgus monkeys. Safety parameters included systemic adverse reactions, including cardiovascular parameters, and haematological and biochemical analysis. Monitoring for systemic adverse reactions through observation of respiratory rate, pulse and temperature was performed. Furthermore, the dogs were sampled for monitoring of complete blood count including platelet count and serum chemistry (urea, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin). The novel *Human-cl rhFVIII* was well tolerated in both dogs. No haematological or biochemical adverse effects were found. There were changes in the haemoglobin concentration, probably related to the blood sampling and CBT assay. No safety issues were detected with *Human-cl rhFVIII*.

Pharmacodynamic drug interactions

Non-clinical PD drug-interaction studies were not performed with *Human-cl rhFVIII*.

2.3.3. Pharmacokinetics

In vivo pharmacokinetic (PK) properties following IV injection were evaluated in a cross-over comparative PD and efficacy study in the haemophilia A dog model (Oct. 10, 2007, OC11-0200). PK properties were also assessed on Day 1 of the repeated-dose toxicity study in Cynomolgus monkeys (DWL0001/063743).

Table 2: Summary of pharmacokinetic studies

Type of study	Species	Administration	Study No.
Pharmacokinetics: FVIII activity	Dog	IV injection	Oct. 10, 2007
Pharmacokinetics: FVIII activity	Dog	IV injection	OC11-0200
Repeated dose toxicity study: Pharmacokinetics assessed on day 1	Monkey	IV injection	DWL0001/063743

In the haemophilia A dog model, PK parameters for *Human-cl rhFVIII* and a BDD rFVIII comparator were in the range expected for this model. In addition, PK properties of *Human-cl rhFVIII,* measured in the preliminary toxicity study in Cynomolgus monkeys, also showed a typical profile expected for a human rFVIII product. The *in vivo* half-lives were between 5.6 and 11 h. The *in vivo* recoveries were between 73% and 87%. The possible effects on endogenous plasma FVIII activity levels by time upon treatment with rhFVIII were also examined and decreased activity was seen in three out of four animals following repeated long-term injections

with rhFVIII. The decreased FVIII:C levels in two of the animals were likely due to the generation of FVIII:C neutralising antibodies caused by the treatment.

No distribution study using radiolabelled compounds or alternative methods has been submitted.

No conventional *in vitro* metabolism studies have been submitted.

The elimination pattern was in line with what is expected for this type of product.

Pharmacokinetic drug interactions

Not applicable.

Other pharmacokinetic studies

Not applicable.

2.3.4. Toxicology

Toxicology studies were performed in rats and monkeys. Acute toxicity was evaluated in a single-dose study in rats. A preliminary toxicity study in Cynomolgus monkeys was performed as a preliminary dose-range finding study, followed by a 28-day main repeated-dose toxicity study. During the main study, a toxicokinetic assessment and a full histopathological analysis were performed. Cardiovascular parameters were evaluated. In the same 28-day study, immunogenicity was evaluated by measuring the formation of anti-FVIII antibodies and FVIII:C neutralising antibodies. The IV administration route was chosen to simulate the conditions of clinical administration. A local tolerance study in rabbits was performed to evaluate local reactions to perivenous administration. Toxicology studies are listed in Table 3. All studies were performed in a good laboratory practice (GLP) compliant manner.

Table 3: Summary of toxicology studies with *Human-cl rhFVIII*

Type of study	Species and strain	Admin.	Duration	Doses (IU/kg)	Study number
Single-dose toxicity	Rat/Crl:CD (SD)IGS BR	IV	Single dose	10,000	DWL0003/ 063496
Repeated-dose toxicity	Cynomolgus monkey	IV	10 doses over 21 days 10 doses over 21 days plus 7 doses at higher dose over 13 days	50 500 followed by 1500	DWL0001/ 063743
	Cynomolgus monkey	IV	28 days	50 or 500	DWL0002/ 064067
Local tolerance	Rabbit/New Zealand White	PV	Single dose	40	DWL0004/073723

IU = international units; IV = intravenous; PV = perivenous.

Single-dose toxicity

Single-dose toxicity study by IV injection to CD rats

Human-cl rhFVIII (10,000 IU FVIII:C/kg body weight [BW] IV) was administered to ten Crl:CD(SD)IGS BR rats (five males and five females). Clinical condition, detailed physical observations and body weight were evaluated. All surviving animals were sacrificed and examined macroscopically at the end of the observation period. Animals were observed for 14 days post-dose. During the observational follow-up period no deaths or in-life changes related to the treatment were recorded and all animals were considered to have achieved satisfactory body weight gain throughout the study. At necropsy one male had a pale liver and one female had congestion of the spleen and pale kidneys that were of not considered to be treatment related.

Repeated-dose toxicity

IV bolus injection to Cynomolgus monkeys

A preliminary toxicity GLP study (DWL0001/063743) in Cynomolgus monkeys was performed to establish an appropriate dosage for the subsequent 28-day repeated toxicity GLP main study (DWL0002/064067). The treatment period was followed by a 14-day observational follow-up period. During the study, test parameters were monitored as follows: clinical condition, body weight, haematology, biochemistry, macroscopic pathology and organ weights. Analyses for clotting function (APTT), FVIII:C levels (chromogenic assay), anti-FVIII antibodies (ELISA assay), and FVIII:C inhibitors (modified Bethesda assay) were also performed. The *Human-cl rhFVIII* test substance was clinically well tolerated and no deaths or any treatment-related adverse effects were recorded in-life and at necropsy. Body weights and biochemistry were not affected by the treatment. The No Observed Adverse Effect Level was > 1500 IU/kg. Based on the results of the preliminary toxicity study, two dosages (a low dose of 50 IU/kg and a high dose of 500 IU/kg [10-fold increase over the clinical dose]) were considered acceptable for a 28-day study assessing the safety of *Human-cl rhFVIII.*

IV administration to Cynomolgus monkey for 4 weeks followed by a 2 week recovery period

The 28-day repeated-dose toxicity study with a 2-week recovery period was performed to assess the systemic toxic potential of *Human-cl rhFVIII* when administered by daily IV injection to Cynomolgus monkeys. A marketed pdFVIII comparator and the formulation vehicle (formulation without active substance) served as controls. FVIII:C, blood clotting times and development of anti-FVIII antibodies were analysed.

Two groups of three male and three female monkeys were intravenously administered with *Human-cl FVIII* with dosages of 50 IU FVIII:C/kg/day and 500 IU FVIII:C/kg/day, respectively. One group of three males and three females was administered with the pdFVIII with a dosage of 500 IU FVIII:C/kg/day. A further control group received the formulation vehicle (formulation buffer of *Human-cl FVIII* without active substance/protein) at the same frequency. A further two

males and two females were included in each of the control and the high doses groups, and followed for a 2-week recovery period after the 28-day treatment period.

In-life observations included clinical monitoring, body weight, ophthalmic examination, electrocardiogram (ECG), haematology and biochemistry, and urinalysis. Furthermore, blood samples were taken for monitoring of the clotting function (APTT), FVIII:C (CHR assay), anti-FVIII antibodies (ELISA assay) and inhibition of FVIII:C (modified Bethesda assay). Histopathology investigation of organs and tissues was also included into the study.

One female animal on high dose, 500 IU FVIII:C/kg *Human-cl rhFVIII*, was sacrificed on Day 30 due to internal bleeding arising from neutralisation of endogenous FVIII. Bruising and haemorrhage occurred at injection sites in a few other animals in the high dose *Human-cl rhFVIII* group. Body weight, ECG, ophthalmoscopy and clinical pathology were not affected by the treatment.

There were no clear treatment effects on blood chemistry parameters.

Necropsy findings in some animals, mainly in the high-dose group, such as pale liver, subcutaneous and muscular haemorrhage, higher spleen weight and low thymus weights, were considered to be possibly related to the haemorrhagic conditions. No evidence of systemic toxicity was recorded. The lower urinary pH and changes noted at necropsy in the thymus (low weight and small appearance) and liver (pallor) were not associated with any histopathological changes. Therefore, these changes were considered of uncertain relationship to treatment and not to be of any toxicological relevance. The 'No Observed Effect Level' was 50 IU/kg.

Genotoxicity

No genotoxicity studies were submitted.

Carcinogenicity

No carcinogenicity studies were submitted.

Reproduction toxicity

No reproductive toxicity studies were submitted.

Local tolerance

Local tolerance of perivenous injections of *Human-cl rhFVIII* was assessed in a GLP study in rabbits (Study No. DWL0004/073723). A dose of 0.2 mL *Human-cl rhFVIII*, at a concentration of 200 IU FVIII:C/mL, was injected over about a 10-second period alongside the left lateral ear vein of four rabbits with the contra-lateral vein being similarly dosed with the formulation vehicle. Animals were observed for 4 days and injection site reactions scored, then rabbits were sacrificed and histopathology examination performed on the injection sites.

There were in-life findings of blanching and purple discolouration and microscopic findings of oedema in the area of the injection sites treated with *Human-cl rhFVIII* that were considered to be a result of the administration procedure. There were no adverse reactions observed to perivenous injection of *Human-cl rhFVIII* or the formulation vehicle. There was no death, no effect on body weight and no clinical signs indicative of a reaction to treatment in any animal. No dermal irritation was observed and there were no macroscopic or microscopic abnormalities evident at necropsy.

No other local tolerance studies for the intended IV route were deemed necessary because the other toxicology studies provided sufficient information concerning possible local effects.

Other toxicity studies

Immunogenicity (ex vivo)

Immunogenicity was evaluated in different studies using the non-clinical *ex vivo* T cell assay EpiScreen, which provides an effective technology for predicting T cell immunogenicity by quantifying T cell responses to protein therapeutics.

The results show that the frequency of positive T cell proliferation responses recorded against the peptides was below the background response threshold for the assay, and therefore the FVIII linker sequences do not contain any significant T cell epitopes. Overall the results in this study correlated well with those obtained from the *in silico* analysis where weak major histocompatibility complex (MHC) class II binding peptides corresponded to peptides that stimulated very weak T cell responses.

A second EpiScreen study was carried out to assess the capacity of VWF to directly modulate T cell activation. The results showed that no effect on keyhole limpet haemocyanin- (KLH) -induced T cell proliferation was observed and the VWF did not affect cell viability. It was therefore concluded that the osmolarity of the bulk culture is within the acceptable range, and VWF is suitable for use in the EpiScreen time course T cell assay.

In continuation of the study as described above, FVIII samples were assessed for immunogenic potential in order to determine the capacity to induce T cell responses in the presence and absence of exogenous VWF. Peripheral blood mononuclear cells (PBMC) from a panel of 50 healthy donors were incubated with the FVIII samples (final concentration 100 IU/mL, except a pdFVIII tested at 25 IU/mL) and T cell response was measured using proliferation assays ([3H]-Thymidine uptake) and IL-2 cytokine secretion (ELISpot).

All samples induced positive proliferation responses in one or more donors in the proliferation assay. Treatment with FVIII product ± VWF induced similar pattern of responding donors. The results show that although VWF appears to generally reduce T cell proliferation against FVIII products, some donors still respond to one or more T cell epitopes that are presented by the FVIII products ± VWF.

The addition of VWF to *Human-cl rhFVIII* reduced the number of observed T cell responses significantly. Comparison of the data obtained from the proliferation and IL-2 ELISpot assays showed that the samples induced similar frequencies of positive T cell responses between the assays.

Data from this study shows that addition of exogenous VWF significantly reduced responses for *Human-cl rhFVIII* tested to below the 10% threshold suggesting that complexing with VWF reduces immunogenicity as seen clinically.

Immunotoxicity

Immunogenicity of *Human-cl rhFVIII* was assessed in the canine model of haemophilia A (Oct. 10, 2007) and in preliminary (WL0001/063743) and repeated-dose (DWL0002/064067) toxicity studies in Cynomolgus monkeys.

In the canine study, a single anti-human FVIII inhibitor test was positive for one of the two dogs in the 96 hour post BDD rFVIII comparator sample (11 days after the infusion of *Human-cl rhFVIII*).

In the preliminary Cynomolgus monkey study, one animal in the 50 IU/kg group developed a low inhibitory activity against *Human-cl rhFVIII* by the end of the study, but not against endogenous FVIII, consequently FVIII:C was unaffected. In the 500 IU/kg group, both animals had an immune response by day 25, with titres measured on days 25, 35, 49 and 55, and a peak response at day 35. The endogenous FVIII activity was decreased from day 35 onwards.

In the repeated-dose toxicity study in Cynomolgus monkeys, the immunogenic reaction to high-dose *Human-cl rhFVIII* (500 IU/kg/day) was comparable to that of a pdFVIII comparator after 28 days of administration. Inhibitors were detected in the majority of animals in both groups on Day 29 (6 of 10 animals in the *Human-cl rhFVIII* group and 8 of 10 animals in the pdFVIII comparator group). No inhibitors were detected in the low dose (50 IU/kg/day) *Human-cl rhFVIII* group.

2.3.5. Ecotoxicity / environmental risk assessment

According to the “Guideline on the environmental risk assessment of medical products for human use” substances like amino acids, peptides, proteins, carbohydrates and lipids are exempted from the guideline since they are unlikely to result in significant risk to the environment. Human-cl rhFVIII is a polypeptide and thereby exempted, consequently, an environmental risk assessment is not required.

2.3.6. Discussion on non-clinical aspects

The programme for the non-clinical safety and efficacy testing of *Human-cl rhFVIII* was designed to assess a protein of known pharmacological action that has a comparable mode of activity to pdFVIII. The main focus of the pharmacological investigation was to evaluate the biological activity *in vitro* and the haemostatic efficacy (together with safety pharmacology aspects and PK profile) *in vivo*.

The BDD rFVIII protein is a well-established substance in treatment of haemophilia A, having proven a comparable mode of clinical function to full-length pdFVIII. Therefore, the pharmacological properties were only assessed in one study using a haemophilia A dog model

and within two toxicology studies in Cynomolgus monkeys. The dog study showed that the infusion of both *Human-cl rhFVIII* and the comparator marketed product induced corresponding reductions of the CBT, increases in plasma FVIII levels and decreases in the APTT and WBCT. Thus, the haemostatic efficacy of *Human-cl rhFVIII* in the haemophilia A dog model was comparable to a commercially available FVIII product. As this study only included two animals, no statistical evaluation is possible and no conclusions can be drawn regarding differences between *Human-cl rhFVIII* and a commercially available FVIII product.

In the Cynomolgus monkey studies, total FVIII:C was increased immediately post-dosing of FVIII, particularly in the high-dose *Human-cl rhFVIII* groups. Reductions in APTT were also seen on Day 1.

In animal models, the PK parameters of *Human-cl rhFVIII* and a commercially available FVIII product were comparable.

No conventional *in vitro* metabolism studies have been conducted with *Human-cl rhFVIII*. Such studies are not considered relevant for a biotechnology-derived product because the expected consequence of metabolism is the normal catabolic degradation to small peptides and individual amino acids. Therefore classical biotransformation studies performed for small molecules are not warranted per current regulatory guidance (International Conference on Harmonisation, 2011 [ICH S6 (R1)]; International Conference on Harmonisation, 1997 [ICH S6]).

The non-clinical safety studies showed *Human-cl rhFVIII* to be well tolerated in rats and monkeys at doses up to and including 10,000 IU/kg, respectively, the highest doses tested.

No non-clinical studies investigating genotoxicity, carcinogenicity and reproduction have been performed. According to ICH S6 guideline recommendations, the omission of studies on genotoxicity, carcinogenicity and reproductive and developmental toxicity is justified. Studies of reproductive and developmental toxicity are not considered relevant as *Human-cl rhFVIII* is a replacement protein for use in the treatment of deficiencies.

The local tolerance study was performed in compliance with the requirements of the “Note for guidance on non-clinical local tolerance testing of medicinal products” (CPMP/SWP/ 2145/00 March 2011). No local reaction to the perivenous injection of *Human-cl rhFVIII* or the formulation vehicle was observed in rabbits.

Immunogenicity data for *Human-cl rhFVIII* obtained as part of toxicity study in dogs and Cynomolgus monkeys were comparable to marketed FVIII comparators and were consistent with expected findings in these animal models.

The type and number of toxicity studies are considered sufficient to support the MAA of Nuwiq as recombinant blood coagulation rFVIII. All findings in the single- and repeated-dose toxicity studies in rats and monkeys were anticipated due to the immune response to *Human-cl rhFVIII*. No signs of non-immunogenic toxicity related to *Human-cl rhFVIII* administration were observed.

According to the “Guideline on the environmental risk assessment of medical products for human use” substances like amino acids, peptides, proteins, carbohydrates and lipids are exempted from the guideline since they are unlikely to result in significant risk to the environment. *Human-cl rhFVIII* is a polypeptide and thereby exempted, consequently, an environmental risk assessment is not required.

Most of the studies were used for several purposes (PD, PK and toxicology), which is principally agreed especially considering animal welfare. In total, one preliminary dose range finding study and four further *in vivo* studies were submitted. The generally limited number of animals included did not allow extensive statistical evaluation, so only general conclusions can be drawn. However, since no safety signals were detected, human data are already available and FVIII products are principally well known, the non-clinical programme can be considered as acceptable to support marketing authorisation.

2.3.7. Conclusion on the non-clinical aspects

Non-clinical studies fulfil the requirements to support marketing authorisation of Nuwiq.

2.4. Clinical aspects

2.4.1. Introduction

PK, efficacy and safety have been investigated in clinical studies in adults and paediatric patients, covering PK evaluation, on-demand treatment, prophylaxis and prophylaxis in surgery.

Studies were performed in the USA, Russia, Bulgaria, Romania, Poland, UK, Turkey, France, Austria and Germany.

GCP

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

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

Tabular overview of clinical studies

Table 4: Completed clinical studies conducted with *Human-cl rhFVIII*

Study	Population	Objectives
GENA-01	22 PTPs(≥150 EDs) 12–65 years	Primary: PK Secondary: IVR, immunogenicity, efficacy†, safety
GENA-08	32 PTPs (≥150 EDs) ≥12 years	Primary: Efficacy§ Secondary: IVR, immunogenicity, safety
GENA-03	59 PTPs(≥50 EDs) 2–12 years	Primary: Efficacy‡ Secondary: PK, IVR, immunogenicity, efficacy in surgical prophylaxis, safety
GENA-09	22 PTPs(≥150 EDs) 18–65 years	Primary: PK Secondary: IVR, immunogenicity, efficacy§ and safety
GENA-04	18 PTPs who had completed GENA-09	Primary: Long-term immunogenicity and tolerability Secondary: Long-term IVR and efficacy§

† In the on-demand treatment of BEs and surgical prophylaxis.
§ In prophylactic treatment, the treatment of BEs and surgical prophylaxis.
‡ In prophylactic treatment and the treatment of BEs.
BE = bleeding episode; ED = exposure day; IVR = in vivo recovery; PK = pharmacokinetic; PTP = previously treated patient.

2.4.2. Pharmacokinetics

Clinical pharmacology of *Human-cl rhFVIII* has been assessed in three multicentre, multinational studies GENA-01, GENA-08 and GENA-03, which provide primary PK data. Single-centre studies, GENA-09 and its extension study GENA-04, provide supportive data.

GENA-01, GENA-03 and GENA-09 included a full analysis of PK parameters. Two studies (GENA-01 and GENA-09) used a randomised cross-over design to compare the PK parameters for *Human-cl rhFVIII* with those of a licensed full-length rFVIII concentrate. GENA-01 and GENA-09 also included a second evaluation of PK parameters for *Human-cl rhFVIII* after 6 months. In GENA-03, a paediatric study, PK parameters were determined at study start only, first for the previously used FVIII concentrate (pdFVIII or rFVIII) and subsequently for *Human-cl rhFVIII*. In the remaining two studies, GENA-04 and GENA-08, only in vivo recovery (IVR) data were collected.

Table 5: Overview of studies involving PK investigations for *Human-cl rhFVIII*

	GENA-01	GENA-08	GENA-03	GENA-09	GENA-04
Patients‡	22 PTPs 12–65 years	32 PTPs 18–75 years	59 PTPs 2–12 years (26 for PK)	22 PTPs 18–62 years	18 PTPs who completed GENA-09
PK investigation (including IVR)	At baseline (full-length rFVIII comparator) and 6 months (only *Human-cl rhFVIII*)	–	At baseline (comparator: previous FVIII concentrate)	At baseline (full-length rFVIII comparator) and 6 months (only *Human-cl rhFVIII*)	–
IVR investigation only	At 3 months	At baseline, 3 and 6 months	At baseline†, 3‡ and 6‡ months	At 3 months	At 3 months and then 3 monthly until study end
Immunogenicity	Yes	Yes	Yes	Yes	Yes
Efficacy	Yes	Yes	Yes	Yes	Yes
Safety	Yes	Yes	Yes	Yes	Yes

‡ All patients.
† Patients not participating in the PK phase of the study.
FVIII = coagulation factor VIII; IVR = in vivo recovery; PK = pharmacokinetic; PTP = previously treated patient.

GENA-01 study

Patients and methods

This study enrolled 22 previously treated patients (PTP) between 12 and 65 years. All patients had undergone the baseline PK assessment, for which they were randomised to receive either *Human-cl rhFVIII* followed by a full-length rFVIII or the full-length rFVIII followed by *Human-cl rhFVIII*. In addition to the baseline PK assessment, a 3-month IVR assessment and a 6-month PK assessment were performed for *Human-cl rhFVIII,* only.

Both infusions for the initial PK assessment and for the 6-month PK assessment of *Human-cl rhFVIII* were preceded by a wash-out phase of at least 96 hours. Blood samples for the determination of FVIII plasma levels were taken before infusion and at 15, 30 and 45 minutes and 1, 3, 6, 9, 12, 24, 30 and 48 hours after the end of the infusion. For IVR analysis at 3 months, the washout period was 72 hours and blood samples were taken before infusion and at 15, 30, 45 and 60 minutes after the end of the infusion.

PK results

Patients received a nominal dose of 50 IU/kg and data were analysed using both the OS and the CHR assay. Results are presented for all 22 patients of the PK per-protocol (PK-PP) population.

The actual doses of *Human-cl rhFVIII* administered for the initial PK assessment were 58.3±3.7 IU/kg as determined by the CHR assay and 48.6±3.3 IU/kg as determined by the OS assay. Respective data adjusted for dose are summarised in the Table 6.

Table 6: GENA-01 study PK results (mean±SD) (PK-PP population, N=22)

Parameter	Assay	*Human-cl rhFVIII*
AUC (h·IU/mL)	CHR	22.5 ± 7.8
	OS	18.0 ± 5.6
AUC_{norm} (h•IU/mL/[IU/kg])	CHR	0.39 ± 0.14
	OS	0.37 ± 0.11
C_{max} (IU/mL)	CHR	1.46 ± 0.22
	OS	1.05 ± 0.15
$C_{maxnorm}$ (IU/mL/[IU/kg])	CHR	0.025 ± 0.004
	OS	0.022 ± 0.003
IVR (% per IU/kg)	CHR	2.50 ± 0.37
	OS	2.14 ± 0.27
T_{max} (h)	CHR	0.35 ± 0.23
	OS	0.43 ± 0.28
$T_{1/2}$ (h)	CHR	14.7 ± 10.0
	OS	17.1 ± 11.2
MRT (h)	CHR	19.5 ± 12.0
	OS	22.5 ± 14.2
CL (mL/h/kg)	CHR	2.94 ± 1.18
	OS	2.96 ± 0.97
V_{ss} (mL/kg)	CHR	49.6 ± 17.3
	OS	59.8 ± 19.8

AUC = area under the curve; AUC_{norm} = area under the curve normalised to the dose administered; CHR = chromogenic; CL = clearance; C_{max} = maximum plasma concentration; $C_{maxnorm}$ = maximum plasma concentration normalised to the dose administered; IU = international units; IVR = in vivo recovery; MRT = mean residence time; N = number of patients; OS = one-stage; PK = pharmacokinetic; PP = per-protocol; SD = standard deviation; $T_{1/2}$ = half-life; T_{max} = time to maximum plasma concentration; V_{ss} = volume of distribution at steady state.

PK profile of *Human-cl rhFVIII* at 6 months was generally comparable to study start, with the exception of outliers from the Bulgarian center.

GENA-08 Study

Patients and Methods

This study enrolled 32 PTPs of at least 12 years of age (actual age range 18–75 years). The IVR of *Human-cl rhFVIII* was assessed at baseline (visit 1) and after 3 and 6 months of treatment. The mean total Haemophilia Joint Health Score (HJHS) in this patient population at study start was 34.6 and the median score was 20.5.

IVR was calculated from the FVIII plasma level pre-infusion and the peak level obtained in the 15, 30, 45 or 60 minutes post-infusion samples. The wash-out periods as required per protocol were at least 72 hours for the baseline investigation and at least 48 hours for the investigations at 3 and 6 months.

IVR Results Over Time

The actual doses of *Human-cl rhFVIII* administered for IVR assessment according to the CHR assay as measured by the central laboratory, at visit 1, 3 months and 6 months were 55.6±2.8 IU/kg, 53.5±4.6 IU/kg and 53.6±2.3 IU/kg, respectively. The corresponding actual doses according to the OS assay were 47.8±2.8 IU/kg, 45.1±4.2 IU/kg and 45.3±2.5 IU/kg.

For both assays, the FVIII:C profiles were highly similar for all three assessments, and there were no marked differences in either peak concentrations or the rate of decrease in FVIII concentration between 15 and 60 minutes across assessments; however, both mean and median IVRs were slightly lower at 3 and 6 months compared with baseline. Both FVIII:C and IVR values based on the OS assay were lower than those obtained with the CHR assay. An analysis of the geometric means of the ratios of individual IVRs was provided, in addition, which was within the accepted bioequivalence range of 80% to 125%.

Absorption, Distribution, Elimination, Metabolism

Not submitted, N/ A (see discussion on Clinical Pharmacology).

***Special populations* - GENA-03 study (paediatric study)**

Patients and methods

This study enrolled 59 PTPs (29 aged 2–5 years and 30 aged 6–12 years). Twenty-seven (26 evaluable) patients participated in the PK phase of the study in which they received their previous FVIII concentrate (pdFVIII or full-length rFVIII) followed by *Human-cl rhFVIII*. An additional 32 patients did not participate in the PK phase, but had an initial IVR assessment. IVR assessments were also performed after 3 and 6 months of treatment in all patients.

Blood samples for the determination of FVIII plasma levels were taken before infusion and 30 minutes and 2, 5, 10, 24 and 48 hours after the end of the infusion.

This study enrolled 59 patients (29 aged 2–5 years and 30 aged 6–12 years). Twenty-seven (26 evaluable) patients participated in the PK phase of the study in which they received their

previous FVIII concentrate (pdFVIII or full-length rFVIII) followed by *Human-cl rhFVIII*. An additional 32 patients did not participate in the PK phase, but had an initial IVR assessment. IVR assessments were also performed after 3 and 6 months of treatment in all patients.

Both infusions for the initial PK assessment were preceded by a wash-out phase of at least 72 hours. Blood samples for the determination of FVIII plasma levels were taken before infusion and 30 minutes and 2, 5, 10, 24 and 48 hours after the end of the infusion.

The IVR of *Human-cl rhFVIII* was calculated after a wash-out period of at least 72 hours from the FVIII plasma level pre-infusion and the peak level obtained in the 30 minutes or 2 hours post-infusion samples.

PK results

Results are presented for all 26 patients of the PK-PP population; separate analyses for patients between 2 and 5 years (N=13) and between 6 and 12 years (N=13) are also included. The actual doses of *Human-cl rhFVIII* administered were 53.1±1.5 IU/kg as determined by the CHR assay and 45.4±1.1 IU/kg as determined by the OS assay. PK results are shown below in Tables 7 and 8.

Table 7: PK parameters for *Human-cl rhFVIII* (dose: 50 IU/kg) in previously treated children aged 2 to 5 years with severe haemophilia A (N=13, dose adjusted)

PK parameter	Assay, mean ± SD	
	Chromogenic	One-stage
AUC (hr*IU/mL)	11.7 ± 5.3	10.1 ± 4.6
$T_{1/2}$ (hr)	9.5 ± 3.3	11.9 ± 5.4
IVR (%/IU/kg)	1.9 ± 0.3	1.6 ± 0.2
CL (mL/hr/kg)	5.4 ± 2.4	5.4 ± 2.3

AUC = area under the curve (FVIII:C); CL = clearance; IVR = incremental *in vivo* recovery; SD = standard deviation; $T_{1/2}$ = terminal half-life.

Table 8: PK parameters for *Human-cl rhFVIII* (dose: 50 IU/kg) in previously treated children aged 6 to 12 years with severe haemophilia A (N=13, dose adjusted)

PK parameter	Assay, mean ± SD	
	Chromogenic*	One-stage
AUC (hr*IU/mL)	13.2 ± 3.4	11.8 ± 2.7
$T_{1/2}$ (hr)	10.0 ± 1.9	13.1 ± 2.6
IVR (%/IU/kg)	1.9 ± 0.4	1.6 ± 0.4
CL (mL/hr/kg)	4.3 ± 1.2	4.1 ± 0.9

*N=12.
AUC = area under the curve (FVIII:C); CL = clearance; IVR = incremental *in vivo* recovery; SD = standard deviation; $T_{1/2}$ = terminal half-life.

***Supportive studies - GENA-09* and GENA-04**

Patients and Methods

GENA-09 enrolled 22 PTPs between 18 and 62 years at a single centre in Russia. This patient population differs from other adult populations in *Human-cl rhFVIII* studies in that these patients had been inadequately treated in the past, as evidenced by the high total HJHS at study start (mean 45.3, median 45.0). Patients were randomised to receive either *Human-cl rhFVIII* followed by a full length rFVIII or the full-length rFVIII followed by *Human-cl rhFVIII* for the initial PK assessment. Patients subsequently received prophylaxis treatment with *Human-cl rhFVIII*, every other day, for 6 months. After 3 months, IVR of *Human-cl rhFVIII* was determined. A second PK assessment (*Human-cl rhFVIII* only) was performed after 6 months.

GENA-04 was the extension study GENA-09. Of the 22 patients enrolled in the parent study, 18 enrolled in the extension study. IVR was assessed at 3 months and subsequently every 3 months until study completion. The study was originally planned to continue until *Human-cl rhFVIII* was registered and launched in Russia.

PK-results

PK parameters were broadly comparable between the two products. When compared with the results of GENA-01 (adult population), the PK results of GENA-09 represent increased "FVIII-turnover" with higher clearance (CL) and lower area under the curve (AUC), maximum plasma concentration (C_{max}), IVR and $T_{1/2}$. However, data reflect a single-centre study, which has been conducted in a region with presumably reduced health care not comparable with Western European countries and has been conducted with a differing population of highly impaired health status.

Mean and median IVRs were comparable for both assays at study start (GENA-09); at 3 and 6 months, values obtained with the OS assay were lower than those obtained with the CHR assay. Nevertheless, the results confirm no marked changes in IVR of *Human-cl rhFVIII* over time.

For GENA-04 results of IVR over time were stable.

Special populations: Increased body weight

Body mass index (BMI) has been identified to affect PK-results and consecutively posology. BMI-dependent PK-results for adults are presented in Table 9.

Table 9: Weight-adjusted PK parameters for *Human-cl rhFVIII* (dose: 50 IU/kg) in adult PTPs (age 18-65 years) with severe haemophilia A (N=20)

PK parameter	All (N=20)	Normal weight (N=14)	Pre-adipose (N=4)	Adipose (N=2)
	Chromogenic assay, Mean ± SD			
AUC (hr*IU/mL)	22.6 ± 8.0	20.4 ± 6.9	24.9 ± 8.9	33.5 ± 6.5
$T_{1/2}$ (hr)	14.7 ± 10.4	14.7 ± 12.1	13.4 ± 5.9	17.2 ± 4.8
IVR (%/IU/kg)	2.5 ± 0.4	2.4 ± 0.4	2.7 ± 0.4	2.8 ± 0.3
CL (mL/hr/kg)	3.0 ± 1.2	3.2 ± 1.3	2.6 ± 1.0	1.8 ± 0.4

PK parameter	All (N=20)	Normal weight (N=14)	Pre-adipose (N=4)	Adipose (N=2)
	Chromogenic assay, Median (range)			
AUC (hr*IU/mL)	22.3 (8.4–38.1)	21.2 (8.4–32.6)	23.3 (17.4–35.5)	33.5 (28.9–38.1)
$T_{1/2}$ (hr)	12.5 (5.4–55.6)	12.3 (5.4–55.6)	11.2 (9.3–22.0)	17.2 (13.8–20.6)
IVR (%/IU/kg)	2.5 (1.7–3.2)	2.4 (1.7–3.1)	2.8 (2.3–3.2)	2.8 (2.6–3.0)
CL (mL/hr/kg)	2.7 (1.5–6.4)	2.8 (1.7–6.4)	2.5 (1.6–3.7)	1.8 (1.5–2.0)

Normal weight: BMI 18.5–25 kg/m^2, pre-adipose: BMI 25–30 kg/m^2, adipose: BMI > 30 kg/m^2.
AUC = area under the curve (FVIII:C); BMI = body mass index; CL = clearance; IVR = incremental *in vivo* recovery; SD = standard deviation; $T_{1/2}$ = terminal half-life.

2.4.3. Pharmacodynamics

Not applicable.

2.4.4. Discussion on clinical pharmacology

Clinical pharmacodynamic studies are not applicable as per the Guideline, nor do they seem meaningful since pharmacologic properties known or thought to be related to the desired clinical effects (biomarkers) and other properties not related to the desired clinical effect of FVIII are not applicable.

Absorption studies are not applicable as per the IV route of administration; distribution, elimination and metabolism studies are not required in accordance with the guideline for the type of product.

Pivotal PK-study GENA-01 in adolescents and adults was provided. In general, the study follows the currently valid Clinical Guideline with regard to study design and patient numbers: Overall, 22 patients between 12 and 65 years of age were included. Numbers, dosage, PK parameters and sampling points were chosen according to the guideline. PK parameters were provided in comparison with a full-length rFVIII and determined by OS and CHR assays. Re-test PK for *Human-cl rhFVIII* was done after 6 months interval. The PK profile of *Human-cl rhFVIII* at 6 months was generally comparable to study start, with the exception of results of outliers from the Bulgarian center.

Overall repeat-PK data are considered to support efficacy of *Human-cl rhFVIII*. Furthermore, IVR-results over time were provided from study GENA-08 covering 32 PTPs 18-75 years of age.

Overall in GENA-01, CHR assay PK parameters for *Human-cl rhFVIII* at a dose of 50 IU/kg in adult PTPs (age 18–65 years) with severe haemophilia A (N=20) were; AUC (hr*IU/mL) 22.6 ± 8.0 [22.3 (8.4–38.1)], $T_{1/2}$ (hr) 14.7 ± 10.4 [12.5 (5.4–55.6)] IVR (%/IU/kg) 2.5 ± 0.4 [2.5 (1.7–3.2)]; and CL (mL/hr/kg) 3.0 ± 1.2 [2.7 (1.5-6.4)].

PK parameters are also presented in the SmPC as an analysis with respect to BMI; as per normal weight: BMI 18.5–25 kg/m^2, pre-adipose: BMI 25–30 kg/m^2, adipose: BMI >30 kg/m^2. Body mass index (BMI) has been identified to affect PK-results and consecutively posology.

BMI-dependent evaluation of PK parameters from studies GENA-01 and GENA-03 was performed. BMI-dependent trends (increasing AUC, $T_{½}$, IVR and decreasing CL follow the assumption of FVIII being active in the intravascular space, mainly. As illustrated, BMI significantly changes PK parameters in haemophilia patients. It is agreed, that the low number of evaluated data-sets does not allow representative conclusions. For overweight patients (documented mainly for the adult population), over-dosing has to be assumed when dosage is based simply upon body weight as addressed in the SmPC. Moreover, a significant part of the paediatric population might be under-dosed with potential "artificially" reduced $T_{½}$ and increased dosing frequency. Consequently, the issue was reflected in the SmPC with respect to posology (dose and frequency of administration - Section 4.2).

Three subjects (ages 12, 12 and 14 years) have been included from the adolescent age-group.

Paediatric population

PK data from a paediatric study (GENA-03) was provided. In general, the study follows the currently valid Clinical Guideline with regard to patient numbers (overall 26 PP-PK patients, 13 in each age-group), dosage, PK parameters and sampling points. The latter slightly deviate from the 4 sampling points of the guideline (baseline, 1 hour, 10 hours, 24 hours and 48 hours after infusion) and reflect baseline, 30 minutes and 2, 5, 10, 24 and 48 hours after the end of the infusion. Due to the known PK acceleration in paediatric age-groups the chosen adaptation is acknowledged.

PK parameters were provided in comparison with previously used FVIII product. No relevant discrepancies were detected. Mean FVIII:C profiles over time, after standardising for actual dose for both products and according to both assays, were provided. Overall, all curves followed a similar pattern.

As known from the literature, IVR and $T_{1/2}$ is lower in young children than in adults and CL is higher, which may be due in part to the known higher plasma volume per kilogram body weight in younger patients.

Comparison of PK results between both age-subgroups, a tendency to reduced AUC, C_{max} and $T_{1/2}$, together with increased CL, has been documented with decreasing age. This phenomenon is well known for similar factor substitutes. Results of IVR over time were stable.

Other special populations

In addition, data from a further study (GENA-09) were provided. This single-centre study comprised 22 patients and was conducted in a region of presumably reduced health care not comparable with Western European countries. Results of that study are therefore only considered to be supportive.

When compared with the results of GENA-01 (adult population), the PK results of GENA-09 represent increased "FVIII-turnover" with higher CL and lower AUC, C_{max}, IVR and $T_{1/2}$. This might be attributable to the impaired overall health status, e.g. highly relevant target joints in the GENA-09 study population.

GENA-04 was an extension study of GENA-09 providing stable IVR-results over time.

Study design and results of the provided pivotal PK studies in adults and paediatric patients as well as supportive data from a special population are considered to adequately confirm an acceptable PK profile of *Human-cl rhFVIII*, in general.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology aspects of *Human-cl rhFVIII* are adequately addressed and fulfil the requirements to support marketing authorisation of Nuwiq. The results including BMI-dependent PK results have been reflected within the SmPC.

2.5. Clinical efficacy

2.5.1. Dose response studies

No dose–response studies have been included in the MAA.

2.5.2. Main studies

See Table 4.

Study GENA-01

Methods

Study GENA-01 was a prospective, actively controlled, open-label cross-over, multicentre phase II study. Patients who completed Part I (see Clinical Pharmacology) were then to be treated for a period of at least 6 months or until 50 exposure days (EDs) were reached, whichever came last (Part II).

Study participants

Inclusion criteria were:

- Severe haemophilia A (FVIII:C ≤1%; historical value as documented in patient records).
- Male patients ≥12 and ≤65 years of age, body weight 25 kg to 110 kg.
- Previously treated with FVIII concentrate, at least 150 EDs.
- Immunocompetent (CD4+ count >200/µL).

- Negative for anti-human immunodeficiency virus (HIV); if positive, viral load less than 200 particles/µL; 400,000 copies/mL.
- Informed consent.

Exclusion criteria were:

- Other coagulation disorder than haemophilia A.
- Present or past FVIII inhibitor activity (≥0.6 BU).
- Severe liver or kidney disease (alanine aminotransferase [ALT] and aspartate aminotransferase [AST] levels >5 times of upper limit of normal, creatinine >120 µmol/L).
- Receiving or scheduled to receive immuno-modulating drugs (other than antiretroviral chemotherapy) such as alpha-interferon, prednisone (equivalent to >10 mg/day), or similar drugs.
- Participation in another interventional clinical study currently or during the past month.

Treatments

Human-cl rhFVIII was administered for treatment of BEs or in case of surgical procedures as an IV bolus injection at a maximum speed of 4 mL/minute. The required dosage was determined using the following formula:

Required units = BW (kg) * desired FVIII rise (%) (IU/dL) * 0.5 (assuming that the IVR of FVIII is 2%/[IU/kg])

The required target peak levels were approximately 40–60% in case of minor haemorrhage; 60–80% in case of moderate to major haemorrhage; 100–120% in case of major to life threatening BEs.

Recommended definitions and dosages for haemorrhages are given in Table 10.

Table 10: Recommended definitions and dosages for haemorrhages

Haemorrhage	Definition	Dosage for BE
Minor	Superficial muscle or soft tissue and oral bleeds	20–30 IU FVIII/kg every 12–24 hours until BE resolution
Moderate to major	Haemorrhage into muscles, into oral cavity; haemarthrosis; known trauma	30–40 IU FVIII/kg, repeated every 12–24 hours until BE resolution
Major to life threatening	Intracranial, intra-abdominal, gastro-intestinal or intra-thoracic bleeds, central nervous system bleeds, bleeding in retropharyngeal spaces or iliopsoas sheath, eyes/retina, fractures or head trauma	An initial dose of 50–60 IU FVIII/kg and subsequently a dose of 20–25 IU FVIII/kg every 8–12 hours until BE resolution

BE = bleeding episode; IU = international units.

The dosage and duration of treatment for surgical prophylaxis with *Human-cl rhFVIII* depended on the type of surgery and the patient's individual incremental IVR. The required dosage was determined using the following formula:

Dose = target increase of FVIII (IU/dL) * BW/actual IVR (IU/dL)/(IU/kg).

Additionally the following dosages were recommended:

Minor surgeries including tooth extractions: 25–30 IU FVIII/kg within 3 hours prior to surgery to achieve an intended target peak level of about 50–60%, repeated every 12−24 hours until healing was complete. Trough level was to be maintained at approximately 30% (samples taken prior to the next infusion of the investigational medicinal product (IMP)).

Major surgeries: 50 IU FVIII/kg within 3 hours prior to surgery to achieve an intended target peak level of approximately 100%, repeated if necessary after 6–12 hours initially and subsequently for at least 6 days until healing was complete. Trough levels were to be maintained at approximately 50% (samples taken prior to the next infusion of the IMP).

Objectives

The primary objective of this clinical study was to determine the PK profile of *Human-cl rhFVIII* in terms of FVIII:C and to compare it with a licensed rFVIII concentrate in PTPs with severe haemophilia A.

Secondary objectives of this study were to:

• Calculate the incremental IVR of FVIII:C for *Human-cl rhFVIII.*

• Investigate the immunogenic potential of *Human-cl rhFVIII.*

• Assess clinical efficacy and safety of *Human-cl rhFVIII* in the treatment of BEs.

• Assess clinical efficacy and safety of *Human-cl rhFVIII* in surgical prophylaxis.

Outcomes/endpoints

After each infusion of IMP and at the end of a BE, the following efficacy assessment was made by the patient (together with the investigator in case of on-site treatment):

• Excellent: Abrupt pain relief and/or unequivocal improvement in objective signs of a BE within approximately 8 hours after a single infusion.

• Good: Definite pain relief and/or improvement in signs of a BE within approximately 8 to 12 hours after an infusion requiring up to 2 infusions for complete resolution.

• Moderate: Probable or slight beneficial effect within approximately 12 hours after the first infusion requiring more than two infusions for complete resolution.

• None: No improvement within 12 hours, or worsening of symptoms, requiring more than 2 infusions for complete resolution.

- **Sample size**

It was planned to include a total of 20 patients in the study. After a further protocol amendment the following was added: Assuming independent binomially distributed success within subjects and centres, the expected ~1000 bleeding episodes in this study can serve to show that the rate of successful treatments (haemostatic efficacy rated good or excellent) is above 70 % (α=0.025, one sided) if the true success rate is 75% or better with a power of 94%. For the secondary hypothesis, 1000 bleedings can serve to show that the rate of successful treatments is above 80 % (α=0.025, one sided) if the true success rate is 85% or better with a power of 98.6%.

- **Randomisation**

This was not applicable as this was a single-arm study.

- **Blinding (masking)**

Not applicable.

- **Statistical methods**

A Statistical Analysis Plan (SAP) was compiled where the statistical analysis of the primary, secondary and safety endpoints was to be understood in the exploratory sense. No confirmatory hypothesis testing was planned.

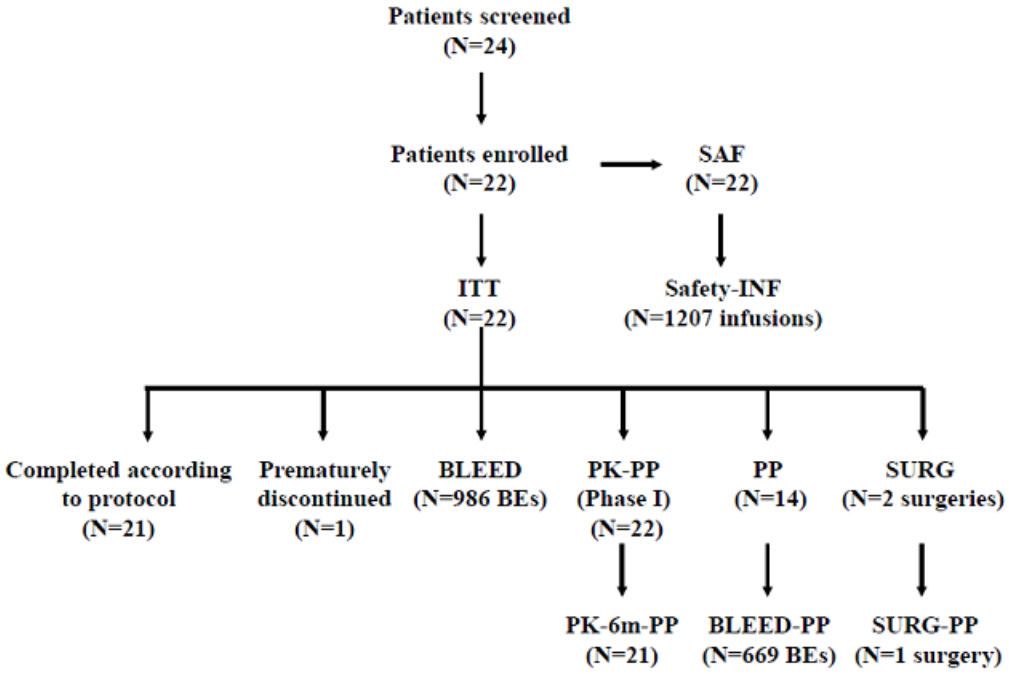
The BLEED population (all BEs documented in the intention-to-treat [ITT] population which received any amount of *Human-cl rhFVIII*) was used to assess the efficacy in the treatment of BEs; The SURG population (documented surgical procedure in the ITT population which used *Human-cl rhFVIII*) was used to assess the efficacy of surgical prophylaxis.

Results

- **Participant flow**

A summary of patient disposition in GENA-01 is shown in Figure 2.

Figure 2: Patient disposition



BE = bleeding episode; BLEED = study population of BEs treated with *Human-cl rhFVIII*; ITT = intention-to-treat; PK = study population of patients undergoing pharmacokinetic analysis; PK-6m = study population of patients undergoing pharmacokinetic analysis at 6 months; PP = per-protocol; SAF = study population of patients in safety analysis; Safety-INF = study population of *Human-cl rhFVIII* infusions; SURG = study population of surgeries treated with *Human-cl rhFVIII*.

- **Conduct of the study**

The protocol version at the time of study start (v. 7, dated 16-Mar-2010) included amendments 1 to 3, during the study 3 further amendments were made. All 22 patients had a least one minor protocol deviation. Four patients used other FVIII after the start of the on-demand phase (major protocol deviations). Most minor protocol deviations were due to not respecting the 72-hour wash-out period, which occurred in 17 patients overall (77.3%).

- **Baseline data**

Main historical BE sites were the ankle (54.5% of patients both sides, 9.1% each for left and right ankle), the knee (45.5% of patients both sides, 9.1% and 22.7% left and right knee, respectively) and the elbow (40.9% of patients both sides, 13.6% and 27.3% left and right elbow, respectively).

Full information on the total number of EDs for previous FVIII concentrates per patient was available for 11 of 22 patients. Four of these received pdFVIII concentrates with a mean of 26.0 EDs and 7 received rFVIII concentrates with a mean of 17.9 EDs within 6 months before the study start.

Six patients were HIV-positive, all with CD4+ T cell counts higher than 200/µL and the HIV viral load as measured by polymerase chain reaction (PCR) well below 400,000 copies/mL.

Baseline demographics and clinical characteristics of patients in GENA-01 are shown in Table 11.

Table 11: Demographic characteristics of study population (ITT/SAF population, N=22)

Parameter	Mean	SD	Median	Range
Age (years)	39.6	14.06	41.0	12–65
Height (cm)	174.0	9.41	176.0	154–188
Weight (kg)	72.7	15.55	69.5	46–105
BMI (kg/m^2)	23.9	4.79	23.0	19–36
HJHS (Gait)	1.6	1.59	1	0–4
HJHS (Total)	38.4	30.29	31.5	0–84
Parameter			**N**	**Percentage**
Race	American Indian or Alaska Native		1	4.5
	White		18	81.8
	Black or African American		3	13.6
Gene defect	Intron 22-inversion		1	4.5
	Inversion type II		1	4.5
	Large deletion/insertion		1	4.5
	Stop mutation		1	4.5
	Unknown		18	81.8
Family history of haemophilia	Yes		14	63.6
	No		8	36.4
Family history of inhibitors	Yes		2	9.1
	No		20	90.9

HJHS = Haemophilia Joint Health Score; ITT = intention-to-treat; SAF = study population of patients in safety analysis; SD = standard deviation.

- **Numbers analysed**

Efficacy results for *Human-cl rhFVIII* were based on the ITT dataset (N=22). Efficacy of *Human-cl rhFVIII* was assessed in BEs for which any amount of treatment with *Human-cl rhFVIII* was documented and which were experienced by patients from the ITT population between the start of home treatment after PK Cycle 2 and the completion visit (BLEED population, N=986 BEs).

Efficacy during surgery was analysed for surgical procedures of patients from the ITT population for which any amount of *Human-cl rhFVIII* prior to, during or after the surgery was documented and no other FVIII concentrate was documented within 24 hours prior to the surgery (SURG population, N=2 surgeries). All subjects in the ITT analysis population who completed the trial without violating the inclusion/exclusion criteria or other aspects of the protocol considered to potentially affect the efficacy results were included in the PP population (N=14).

- **Outcomes and estimation**

Bleeding episodes

In total there were 997 BEs that started after start of home treatment after PK Cycle 2. Thereof, 986 BEs were documented BEs in patients in the ITT population for which any amount of treatment with *Human-cl rhFVIII* was documented and which started between initiation of home treatment after PK cycle 2 (i.e., one day after drawing of last blood sample for PK) and the completion visit.

Of the 986 BEs, 642 (65.1%) were spontaneous, 341 (34.6%) were traumatic, and 3 (0.3%) were due to other causes. In total, 416 (42.2%) were minor, 566 (57.4%) were moderate to major and 3 (0.3%) were major to life-threatening. These 3 BEs are described in more detail below. The severity for one BE was unknown.

The number of BEs treated with *Human-cl rhFVIII* per patient ranged from 15 to 93. The most common sites of bleeding were the knee (230 BEs 23.3%), the elbow (225 BEs, 22.8%), other sites (166 BEs, 16.8%) and the ankle (155 BEs, 15.7%).

Two of the 3 major to life-threatening BEs were traumatic bleeds in one patient; the first BE was treated with 3 infusions on 2 EDs with a total of 7000 IU (104.5 IU/kg) of *Human-cl rhFVIII* with moderate efficacy; the second BE was treated with 2 infusions over 2 EDs with 5500 IU (82.1 IU/kg) of *Human-cl rhFVIII* with good efficacy. The third major to life-threatening BE was a spontaneous right and left groin bleed in patient 010101 which required only 1 infusion of *Human-cl rhFVIII* (4000 IU; 45.5 IU/kg) to treat and the efficacy of that treatment was judged as good.

A summary of the EDs and dosages with *Human-cl rhFVIII* for the treatment of BEs is shown in Table 12.

Table 12: EDs and *Human-cl rhFVIII* dosages for treatment of BEs (BLEED population, N=986)

Parameter	Mean	SD	Median	Range
Number of infusions per bleeding site*	1.1	0.59	1.0	1-13
Dose of *Human-cl rhFVIII* per infusion, IU	2375	1055	2000	500-6000
Dose of *Human-cl rhFVIII* per infusion, IU/kg	32.3	10.59	30.0	7-61
Number of EDs per BE*	1.1	0.55	1.0	1-13
Dose of *Human-cl rhFVIII* per BE, IU	2693	2618	2000	500-65,000
Dose of *Human-cl rhFVIII* per BE, IU/kg	36.6	27.64	30.9	8-657

* Dosage used to treat several simultaneous bleedings are counted only once in this analysis.
BE = bleeding episode; BLEED = study population of BEs treated with *Human-cl rhFVIII*; ED = exposure day; IU = international unit; SD = standard deviation.

The mean duration of treatment of BEs overall was 1.1±0.75 days (range 1-19 days). Minor BEs required a mean of 1.0±0.17 days of treatment, moderate to major BEs 1.2±0.96 days and major to life-threatening 1.7±0.58 days. The one BE that was of unknown severity (spontaneous bleed in the right forearm in patient 010501) required 2 days of treatment.

Personal efficacy assessments were used for analysis and were available for 985 BEs and missing for 1. Overall, 94.4% (931/986) of the BEs were treated with excellent or good efficacy (60.3% excellent, 34.1% good). Treatment efficacy was judged as moderate in 5.5% of BEs. In no BE was *Human-cl rhFVIII* treatment judged as having no efficacy.

Efficacy assessment of each individual infusion revealed similar results. Overall, 90.4% (941/1041 infusions of BLEED population) infusions were judged as having excellent or good efficacy (48.2% excellent, 42.2% good). Treatment efficacy was judged as moderate in 88 (8.5%) infusions and as none in 9 (0.9%) of infusions. Efficacy assessment was missing for 3 infusions.

Regarding the 9 infusions that were judged as having no efficacy, they were all administered for moderate to major BEs. Six of these infusions were given to the same patient for 2 BEs due to a trauma, 5 were received for one BE (all at the dose of 34.88 IU/kg), for a total *Human-cl rhFVIII* dose of 15,000 IU (174.4 IU/kg). Three infusions were rated as having no efficacy and 2 as having moderate efficacy. The overall assessment of *Human-cl rhFVIII* efficacy for this BE was moderate. For the second BE the patient received 3 infusions at the dose of 34.88 IU/kg, for a total *Human-cl rhFVIII* dose of 9,000 IU (104.7 IU/kg); the overall assessment of *Human-cl rhFVIII* efficacy for this BE was assessed as good.

The other 3 infusions that were judged as having no efficacy were administered for a single BE; 4 infusions of *Human-cl rhFVIII* over 3 EDs (40.0 IU/kg for all) were administered for a total dose of 11,500 IU (153.3 IU/kg). Efficacy of 3 infusions for this BE was judged as none and for one infusion as moderate, and the overall efficacy of *Human-cl rhFVIII* for this BE was moderate.

Surgery

During the study, 2 patients underwent 2 surgical procedures, one major (revision of right total knee – 15 infusions) and one minor (colonoscopy / oesophagogastrodudenoscopy – 5 infusions). The duration of the minor surgery was 51 minutes and of the major surgery 150 minutes. Efficacy of *Human-cl rhFVIII* during surgeries was assessed intra-operatively by the surgeon and overall by the surgeon and haematologist. Both intra-operative and overall efficacy was rated as excellent for both surgeries.

- **Ancillary analyses**

Not applicable.

- **Summary of main efficacy results**

Study GENA-08

Methods

- **Study participants**

Inclusion and exclusion criteria were as described for GENA-01.

- **Treatments**

Patients being treated prophylactically were to receive 30–40 IU FVIII/kg every other day until 6 months and at least 50 EDs had been reached. Two dose escalations of +5 IU/kg each were allowed in case of an inadequate response (≥2 spontaneous BEs during one month). On-demand treatment and surgical prophylaxis followed the same methodology as described for GENA-01.

- **Objectives**

The primary objective of this clinical study was to determine the efficacy of *Human-cl rhFVIII* during prophylactic treatment, in the treatment of BEs and in surgical prophylaxis in PTPs with severe haemophilia A. Secondary objectives of this study were as described for GENA-01.

- **Outcomes / endpoints**

The primary endpoint was the efficacy of *Human-cl rhFVIII* in prophylaxis, in treatment of BEs and in surgical procedures.

For prophylactic treatment, primary efficacy variables were the overall efficacy assessment after a total of at least 50 EDs at the end of the study and consumption of IMP (FVIII IU/kg per month, per year) per patient and in total.

The frequency of spontaneous breakthrough BEs/month under prophylactic treatment was assessed as excellent, good, moderate or poor:

- Excellent: Less than 0.75 spontaneous BEs per month
- Good: Between 0.75 and 1 spontaneous BEs per month
- Moderate: Between 1 and 1.5 spontaneous BEs per month
- Poor: More than 1.5 spontaneous BEs per month

In addition to the primary analysis of spontaneous BEs, an additional assessment of the monthly BE rate for all types of BE was performed.

For on-demand treatment of BEs, the primary efficacy variable was the efficacy assessment at the end of the BE. Efficacy ratings were the same as described for GENA-01 (excellent, good, moderate and none).

For surgical procedures, primary efficacy variables were the overall efficacy assessment after the end of the surgical prophylactic treatment phase and average and maximum expected estimated blood loss compared to the actual estimated blood loss. Efficacy was assessed at the end of surgery by the surgeon and postoperatively by the surgeon and the haematologist using an objective scale taking into account intra- and post-operative assessments.

An overall efficacy assessment taking both the intra- and post-operative assessment into account was done by the surgeon and haematologist.

- **Sample size**

It was planned to include a total of 32 evaluable patients into the study. No inferential analysis involving formal testing was planned and consequently no formal sample size estimation was performed.

- **Randomisation**

This was not applicable as this was a single-arm study.

- **Blinding (masking)**

This was not applicable as this was an open-label study.

- **Statistical methods**

Statistical study conduct

A SAP was compiled as a separate document. No inferential statistics were planned for this uncontrolled efficacy trial; planned statistics were exploratory and descriptive. The subject disposition, i.e. the identification of significant deviations to consider for the PP population and the assignment of each subject to these analysis populations, was to be the joint decision of the trial statistician and the responsible medical expert in a data review meeting prior to database lock. The final statistical analysis was to be conducted upon completion of all database release procedures (DBR) and sign-off of the DBR release form.

Three populations were considered:

Safety analysis population: All subjects who received at least one dose of *Human-cl rhFVIII*;

Intent to Treat (ITT) analysis population: All subjects in the safety analysis population for whom any data was collected post treatment with *Human-cl rhFVIII*

Per Protocol (PP) analysis population: All subjects in the ITT analysis population who completed the trial without significantly violating the inclusion/exclusion criteria or other aspects of the protocol considered to potentially affect the efficacy results.

In addition to the basic populations the following subpopulations were defined (similar definitions were also made based on the PP population):

Population of subjects on prophylactic treatment schedule (PROPH): All subjects in the ITT population who had at least one prophylactic treatment. The PROPH population was to be identical to the ITT population, if all subjects admitted to the trial underwent prophylactic treatment.

Population of BEs (BLEED): All documented BEs of subjects in the ITT population which received any amount of treatment with *Human-cl rhFVIII*.

Surgery population (SURG): All documented surgical interventions of subjects in the ITT population which received *Human-cl rhFVIII* prior to, during or after the surgery is documented, but did not receive any other FVIII concentrate within 24 hours prior to surgery.

The ITT analysis was considered to be the most relevant for efficacy data. To evaluate the robustness of the study results, efficacy analyses were also to be done on the basis of the PP population.

The primary objective of this clinical study was to determine the efficacy of *Human-cl rhFVIII* in previously treated subjects suffering from severe haemophilia A (FVIII:C ≤1%). This intent was three fold, demonstration of efficacy of prophylactic treatment in the ITT/PROPH population, of treatment of breakthrough bleeds in the BLEED population as well as of treatment in the context of surgery in the SURG population.

Efficacy of Prophylactic Treatment

The efficacy was to be evaluated by descriptive statistics on bleeding rates and was to be presented in summary tables. The frequency of BEs under prophylactic treatment was to be calculated after a total of 50 EDs and at the end of the study in order to assess overall efficacy. The frequency of BEs (overall and by type of BE) under prophylactic treatment was to be calculated using the following definitions for the prophylactic treatment time period:

- The time between first prophylactic treatment with *Human-cl rhFVIII* until the administration of the 50th ED for the 50 ED analysis minus time periods from start of treatment for surgery until restart of every other day prophylactic treatment

- The time between first prophylactic treatment with *Human-cl rhFVIII* until last prophylactic treatment +2 days or study completion whichever comes first minus time periods from start of a surgery until restart of every other day prophylactic treatment.

As per the statistical analysis plan the overall efficacy assessment after a total of 50 EDs and after end of the study was to be assessed by the frequency of spontaneous BEs (i.e. traumatic, post-operative or “Other” BE not included) as the primary analysis for prophylactic treatment. The frequency of traumatic and all (spontaneous, traumatic, other) BEs were defined as secondary analyses.

The frequency of breakthrough BEs/months under prophylactic treatment was to be calculated and assessed as excellent, good, moderate or poor:

Excellent: Less than 0.75 spontaneous BE per month.

Good: Between 0.75 and 1 spontaneous BE per month.

Moderate: Between more than 1 and 1.5 spontaneous BEs per month.

Poor: More than 1.5 spontaneous BEs per month.

Treatment of Breakthrough Bleeding Episodes

The efficacy was to be evaluated by descriptive statistics on treated bleeds.

The clinical efficacy of *Human-cl rhFVIII* in on-demand treatment of breakthrough BEs was to be assessed by analysing the following parameters: Efficacy assessment by the subject at the end of the bleeding episode, together with the investigator in case of on-site treatment (excellent, good, moderate, none). In addition to the four point scale the proportion of BEs successfully treated with *Human-cl rhFVIII* was to be evaluated for all BEs. “Successfully treated” are all “excellent” and “good” efficacy ratings of treated BEs.

Haemostatic control in Surgical Procedures

The efficacy in the context of surgeries was to be evaluated by descriptive statistics.

Efficacy was to be evaluated by the surgeon and the haematologist at the end of the surgery and on the last post-operative day. In addition to the four point scale (very good, good, moderate, none), the proportion of surgeries with successful treatment according to the overall efficacy

assessment was to be presented. “Successfully treated” was defined as all “excellent” and “good” efficacy ratings of surgical procedures.

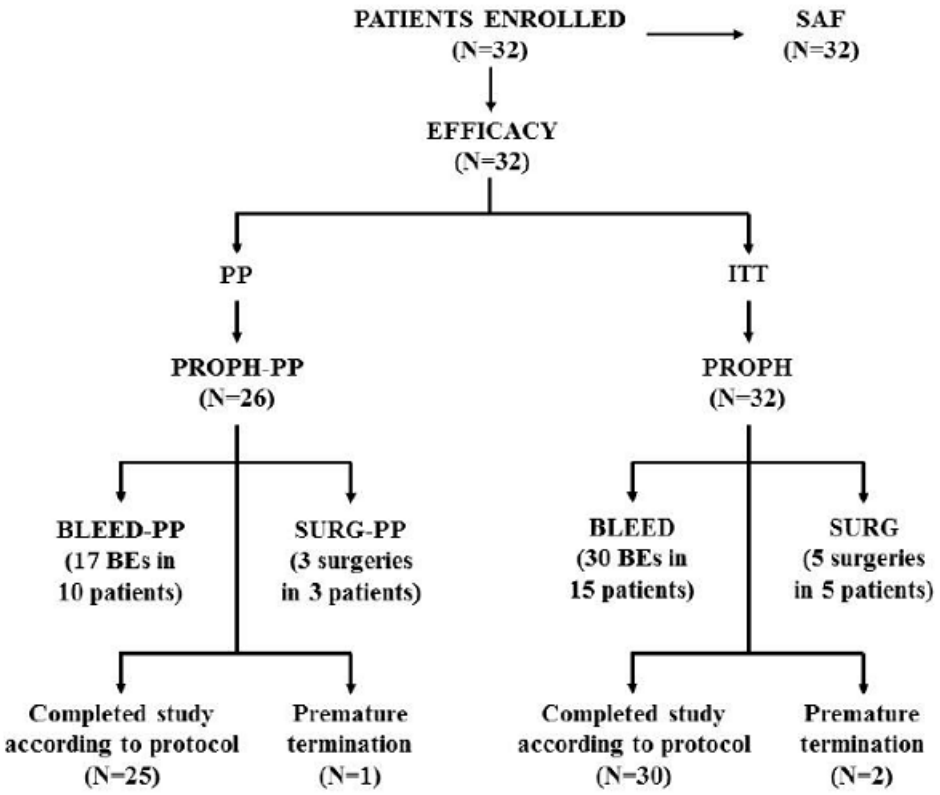
No missing data imputation was foreseen with the exception of bodyweight, where the last available weight measurement was to be used. Subjects who permanently switched to another FVIII product during their study participation were to be considered treatment failures, i.e. the efficacy was to be imputed to be “none” for each haemostatic efficacy assessment after the switch in the efficacy analyses, unless the infusion of another FVIII concentrate was due to an emergency situation and if the IMP was not available for the patient in time.

Results

Participant flow

Patient disposition in GENA-08 is summarised in Figure 3.

Figure 3: Patient disposition



BE = bleeding episode; BLEED = study population of BEs treated with Human-cl rhFVIII; PROPH = study population of patients receiving prophylaxis; ITT = intention-to-treat; PP = per-protocol; SAF = study population of patients in safety analysis; SURG = study population of surgeries treated with Human-cl rhFVIII.

Recruitment

This study was conducted in 11 investigational centres in Austria, Bulgaria, Germany and the UK. First Patient In: 22-Jun-2010; Last Patient Out: 31-Jan-2012.

Conduct of the study

The protocol version at the time of study start (v. 7, dated 16-Mar-2010) included amendments 1–3, and was further amended to change end of trial from Q1 2011 to Q4 2011 and with other minor changes and clarifications.

Thirty-one patients had a least one minor protocol deviation. Major protocol deviations were recorded in 5 patients.

Baseline data

Baseline demographics and clinical characteristics of patients in GENA-08 are shown in Table 13.

Table 13: Demographic characteristics of study population (ITT/SAF population, N=32)

Parameter	Mean	SD	Median	Range
Age at first treatment (years)*	37.3	13.6	35.0	18–75
Height (cm)	178.4	7.9	180.0	158–192
Weight (kg)	82.5	18.0	84.5	47–127
HJHS (Gait)	1.6	1.4	2.0	0–4
HJHS (Total)	34.6	32.2	20.5	0–117

Parameter		N	Percentage
Race	Asian	3	9.4
	White	29	90.6
Gene defect	Point mutation	1	3.1
	Small deletion/insertion	2	6.3
	Missense mutation	1	3.1
	Disval inversion	1	3.1
	2071< >A	1	3.1
	C.3863 dupC	1	3.1
	764/C.764G>T	1	3.1
	CYS310<FS	1	3.1
	Exon 14 2379–2385 del, 2378 ins CTCTT	1	3.1
	Intron 22 inversion	3	9.4
	Unknown	19	59.4
Family history of haemophilia	Yes	18	56.3
	No	14	43.8
Family history of inhibitors	Yes	1	3.1
	No	30	93.8
	Unknown	1	3.1

* With *Human-cl rhFVIII*.
A = adenine; C = cytosine; CYS = cysteine; del = deletion; FS = frameshift; G = guanine; HJHS = Haemophilia Joint Health Score; ins = insertion; ITT = intention-to-treat; SAF = study population of patients in safety analysis; T = thymine.

FVIII inhibitor levels were less than 0.6 BU in 31 patients at screening. Patient 082101 had a relatively high FVIII:C of 0.649 IU/mL as measured by the OS assay at screening, probably due to an infusion of FVIII 1 to 2 days before as the patient was still receiving prophylaxis with a different FVIII concentrate at that time. Consequently, the results for the inhibitor measurement were reported as indeterminate because a potential low-titre inhibitor may have been masked by the high FVIII:C. The patient had inhibitor titres of less than 0.6 BU in all further measurements.

Main historical BE sites were the ankle (59.4% of patients both sides, 15.6% and 3.1% left and right ankle, respectively), the elbow (40.6% of patients both sides, 12.5% and 21.9% left and right elbow, respectively) and the knee (31.3% of patients both sides, 25.0% and 18.8% left and right knee, respectively).

All 8 patients from the centre in Bulgaria and 3 patients from two UK centres had previously received on-demand treatment with an FVIII concentrate; the remaining 21 patients had received FVIII concentrates as prophylaxis. Overall, pdFVIII concentrates had been used in 10 patients and rFVIII concentrates in 22 patients.

Numbers analysed

Efficacy results for *Human-cl rhFVIII* are based on the ITT dataset. The efficacy of prophylaxis was analysed for all patients who received at least one prophylactic dose of *Human-cl rhFVIII* (PROPH population, N=32). Efficacy of *Human-cl rhFVIII* for the treatment of BEs in patients receiving *Human-cl rhFVIII* as prophylaxis were analysed for all BEs treated with at least one dose of *Human-cl rhFVIII* (BLEED population, 30 BEs in 15 patients). Efficacy during surgery was analysed for surgical procedures where at least one dose of *Human-cl rhFVIII* was administered (SURG population, 5 surgeries in 5 patients).

The SAF population included all patients who received at least one dose of *Human-cl rhFVIII* (N=32); this population is identical to the ITT population.

Outcomes and estimation

Prophylaxis

All 32 patients started prophylactic treatment with *Human-cl rhFVIII* and were included in the PROPH population. The total number of prophylactic injections was 2722.

A summary of the EDs and dosages with *Human-cl rhFVIII* for the treatment of BEs is shown in Table 14 and the frequency of BEs is shown in Table 15.

Table 14: EDs and dosage for prophylatic treatment with *Human-cl rhFVIII* (PROPH population, N=32)

Parameter	Mean	SD	Median	Range
Number of EDs	85.1	15.4	89.0	16–100
Number of infusions per ED	1.0	0.0	1.0	1–1
Duration of prophylactic treatment (months)	6.0	0.9	6.1	1.3–7.3
Total dose of *Human-cl rhFVIII* (IU)	226,596	59,767	228,020	56,000–325,548
Average amount of *Human-cl rhFVIII* per month of study (IU/kg/month)	466.1	65.5	468.7	208.4–582.6
Average dose of *Human-cl rhFVIII* per infusion (IU)	2689	572	2503	1500–4063
Average dose of *Human-cl rhFVIII* per infusion (IU/kg)	32.8	2.8	33.1	24.0–39.3

ED = exposure day; IU = international unit; PROPH = study population of patients receiving prophylaxis; SD = standard deviation.

Table 15: Frequency of BEs during the study (PROPH population, N=32)

Frequency of BEs	N	%	Cumulative %
0	16	50.0	50.0
1	11	34.4	84.4
5	1	3.1	87.5
6	1	3.1	90.6
7	2	6.3	96.9
8	1	3.1	100.0

BE = bleeding episode; PROPH = study population of patients receiving prophylaxis.

The mean total number of BEs during the study was 1.4±2.4 per patient (median 0.5; range 0–8). When excluding BEs occurring under surgical prophylaxis and BEs occurring between the first recovery dosing and start of prophylactic treatment, the mean number of BEs per patient during the study was 0.6±1.2 for spontaneous BEs (median 0; range 0–4) and 1.1±1.8 for all types of BEs (median 0.5; range 0–7).

The mean BE rates per patient during the prophylactic treatment period at the end of the study were 0.095/month for spontaneous BEs (median 0; range 0–0.71) and 0.188/month (median 0.074; range 0–1.21) for all types of BE. Consumption (e.g. 450 IU/month or 5400 IU/year for adults in GENA 08) has been provided and reflected in the SmPC.

In all 11 patients who had received on-demand treatment prior to the study, monthly BE rates were markedly reduced; the mean BE rate in these patients decreased from 3.924 to 0.043.

Eight patients experienced no BEs at all during the study and the remaining three had low monthly BE rates of 0.148–0.166.

In all 32 patients, the overall efficacy for spontaneous BEs was excellent (100%); for all types of BEs efficacy was excellent or good in 31 out of 32 patients (96.9%) and moderate one patient (3.1%).

Treatment of BEs

Out of the 44 BEs occurring during the study, 30 were treated with at least one dose of *Human-cl rhFVIII*. Of the 30 treated BEs, 14 were spontaneous, another 14 were due to trauma, and the remaining two were classified as "other". The most frequent sites of BEs were the ankle and the knee, which together accounted for 60% of all BEs. Personal efficacy assessments were used for analysis and were available for 28 BEs, 14 of which were minor and 14 were moderate to major. Efficacy ratings on a four-point scale were excellent or good for all BEs (Table 16).

Table 16: Personal efficacy assessment for treatment of BEs according to severity (BLEED population, 28 BEs in 15 patients)

Severity of BE (number of BEs) Efficacy rating	N	%
Any (N=28)		
Excellent	20	71.4
Good	8	28.6
Moderate	0	0.0
None	0	0.0
Minor (N=14)		
Excellent	12	85.7
Good	2	14.3
Moderate	0	0.0
None	0	0.0
Moderate to major (N=14)		
Excellent	8	57.1
Good	6	42.9
Moderate	0	0.0
None	0	0.0

BE = bleeding episode; BLEED = study population of BEs treated with *Human-cl rhFVIII*.

For 2 BEs, no personal efficacy assessment was available. At the end of the study, the IDMC adjudicated on all personal efficacy assessments, primarily considering the number of infusions administered for each BE. In six cases the IDMC assessment differed from the personal assessment. In three cases in which the personal assessment had been 'good', efficacy was judged 'moderate'; in two cases in which the personal assessment had been 'good', efficacy was

rated as 'excellent'; and in one case where no personal assessment was available, efficacy was rated as 'excellent' by the IDMC.

Surgery

The SURG population comprised 1 minor and 4 major surgeries in 5 patients. An overview of all surgical procedures included in the analysis is provided in Table 17.

Table 17: Description, blood loss and efficacy rating of surgical procedures (SURG population, 5 surgeries in 5 patients)

Patient	Type of surgery	Description of surgery	Difference between actual and expected average blood loss (mL)	Number of infusions	Total dose (IU/kg)	Overall efficacy rating
080302	Major	Joint arthroscopy	50	25	1028.74	Moderate
081402	Major	Bilateral ankle joint arthroscopic debridements	–20	9	320.92	Excellent
081501	Major	Total hip replacement	–500	16	480.39	Excellent
081701	Major	Cholecystectomy and liver biopsy	N/A	5	183.33	Excellent
082101	Minor	Tooth extraction	N/A	3	95.45	Excellent

IU = international unit; N/A = no data available; SURG = study population of surgeries treated with *Human-cl rhFVIII*.

For 3 surgeries, average and maximum expected and actual blood loss were reported. For 2 procedures with an expected average blood loss of 20 and 500 mL, no actual blood loss was observed; for one surgery, the actual blood loss was 100 mL, which was 50 mL higher than the average expected blood loss, but still markedly below the expected maximum blood loss of 600 mL.

For 4 surgeries (the minor and 3 major procedures), efficacy was rated as excellent intra-operatively and overall. For one major surgery (patient 080302), intra-operative efficacy was rated as good and overall efficacy as moderate.

Information on *Human-cl rhFVIII* dosage is shown in Table 18.

Table 18: Summary of EDs and dosages administered for surgeries (SURG population, 5 surgeries in 5 patients)

Parameter	Mean	SD	Median	Range
Total dose of *Human-cl rhFVIII* (IU)	37,680.0	32,711.4	25,000.0	8400–89,500
Total dose of *Human-cl rhFVIII* (IU/kg)	421.77	369.24	320.92	95.5–1028.7
Pre-operative loading dose (IU/kg)	49.61	6.58	50.00	39.2–57.5
Infusions after end of surgery (IU/kg)	372.16	366.74	269.58	45.5–971.3
Total dose of *Human-cl rhFVIII* per ED (IU/kg)	54.86	13.13	60.05	31.8–64.2
Dose of *Human-cl rhFVIII* per infusion (IU)	3104.06	341.16	3062.50	2777.8–3580.0
Dose of *Human-cl rhFVIII* per infusion (IU/kg)	35.06	4.36	35.66	30.0–41.1

ED = exposure day; IU = international unit; SD = standard deviation; SURG = study population of surgeries treated with *Human-cl rhFVIII*.

Ancillary analyses

Not applicable.

Summary of main efficacy results

Table 19: Summary of efficacy for trial GENA-08

Title: Clinical study to investigate the efficacy, safety, and immunogenicity of *Human-cl rhFVIII* in PTPs with severe haemophilia A		
Study identifier	GENA-08	
Design	Prospective, open-label, international, multicentre Phase 3 study	
	Duration of main phase:	6 months and at least 50 EDs
	Duration of run-in phase:	not applicable
	Duration of extension phase:	not applicable
Hypothesis	Exploratory study to determine the efficacy of *Human-cl rhFVIII* during prophylactic treatment in PTPs with severe haemophilia A, in the treatment of BEs and in surgical prophylaxis.	

Populations	PROPH population		Patients who initiated prophylactic treatment with *Human-cl rhFVIII* (N=32) 30–40 IU FVIII/kg BW every other day until 6 months and at least 50 EDs had been reached. Two dose escalations of +5 IU/kg BW each were allowed in case of an inadequate response (≥2 spontaneous BEs during one month).
	BLEED population		Patients with BEs that were treated with *Human-cl rhFVIII* (N=15, 30 BEs) Dosage and duration for the treatment of BEs depended on the location and extent of bleeding and on the clinical situation of the patient.
	SURG population		Patients who underwent surgical procedures during which *Human-cl rhFVIII* was used (N=5) Dosage and duration of treatment with *Human-cl rhFVIII* depended on the type of surgery and the patient's individual incremental recovery
Endpoints and definitions	Primary endpoint	Efficacy	Efficacy of *Human-cl rhFVIII* during 1) prophylactic treatment, 2) in the treatment of BEs and 3) in surgical prophylaxis.
	Secondary endpoints	IVR	To calculate the incremental IVR of FVIII:C for *Human-cl rhFVIII*
		Immunogenicity	To investigate the immunogenic potential of *Human-cl rhFVIII*
		Safety	To assess the safety of *Human-cl rhFVIII*
Database lock	31 January 2012		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population	ITT		
Efficacy of prophylaxis (N=32)	BE severity during prophylaxis: Minor (63.6%), moderate to major (36.4%) BE type during prophylaxis: Spontaneous (59.1%), traumatic (36.4%), other (4.5%) Efficacy assessment of prophylaxis based on spontaneous BE rates: Excellent (100%) Efficacy assessment of prophylaxis based on all BE rates: Excellent (90.6%), good (6.3%) [96.9% excellent or good], moderate (3.1%)		

Efficacy of the treatment of BEs (N=15, 30 BEs)	Severity of treated BE: Minor (46.7%), moderate to major (53.3%) Efficacy assessment for the treatment of BEs (28 BEs evaluable): Excellent (71.4%), good (28.6%) [100% excellent or good]
Efficacy of surgical prophylaxis (N=5)	Efficacy assessment: Excellent (80%), moderate (20%)
Notes	A total of 65 treatment-emergent AEs were recorded in 21 of the 32 patients (65.6%). Of these, 59 (90.8%) were mild (69.2%) or moderate (21.5%) in severity, and all except one case of exacerbation of arthralgia in a target joint were resolved without sequelae. Six AEs in 4 patients were rated as severe and two of those (occurring in 2 patients) were also rated as serious; one patient died following a *status epilepticus*. These 6 AEs were either due to an accident or could be explained by the patients' medical history; none were deemed related to *Human-cl rhFVIII* administration. Two patients experienced a total of 5 possibly related AEs. One patient reported injection site pain after the first infusion; the second patient experienced vertigo, dry mouth and paraesthesia after the first and injection site inflammation after the 15th administration of *Human-cl rhFVIII*. All of these 5 AEs were mild, non-serious and fully resolved without requiring any action.
Analysis description	**Statistical Analysis**
	The statistical analysis of all endpoints was exploratory. No confirmatory hypothesis testing was planned. Due to the limited number of patients, no stratification for any subgroup analyses was performed, except for the analysis of the subgroups of patients with BEs and those with surgeries.

AE = adverse event; BE = bleeding episode; BW = body weight; ED = exposure day; FVIII:C = FVIII coagulant activity; IVR = *in vivo* recovery; ITT = intention-to-treat; PTP = previously treated patient.

GENA-03

Methods

Study participants

The study population were paediatric PTPs (age ≥2 and 12 years) with severe haemophilia A (FVIII:C <1%) with at least 50 EDs to their current FVIII product. Patients were immunocompetent (CD4+ count >200/µL); HIV-negative or respective viral load <200 particles/µL or <400,000 copies/mL; and written informed consent obtained.

Exclusion criteria included: Other coagulation disorder than haemophilia A; present or past FVIII inhibitor activity (≥0.6 BU); target joints; severe liver or kidney disease (ALT and AST levels >5 times of upper limit of normal, creatinine >120 µmol/L); receipt or scheduled receipt of immuno-modulating drugs (other than antiretroviral chemotherapy) such as alpha-interferon, prednisone (>10 mg/day), or comparable drugs; current participation in another clinical study.

Treatments

For prophylactic treatment in Phase II, 30–40 IU FVIII/kg BW of *Human-cl rhFVIII* were administered every other day or 3 times weekly until 6 months and ≥50 EDs had been fulfilled. Two dose escalations of each +5 IU/kg BW were allowed in case of an inadequate response (≥2 spontaneous BEs within one month). The dosage and duration of treating spontaneous or traumatic breakthrough BEs within the prophylactic treatment period and during surgeries depended both on the location and on the extent of the BE, and on the clinical condition of the respective patient. The required dosage and target peak plasma levels were the same as described for GENA-08. The required dosage and the dosing recommendations given for surgical prophylaxis were the same as described for GENA-01.

Objectives

The primary objective was to assess clinical efficacy of *Human-cl rhFVIII* in terms of prevention (prophylactic treatment) and treatment of (breakthrough) BEs in previously treated children suffering from severe haemophilia A (FVIII:C <1%).

Secondary objectives of this trial were:

- To determine PK parameters (in up to 13 patients [12 evaluable] of each age group, 2 to 5 and 6 to 12 years) of *Human-cl rhFVIII.*
- To determine the incremental IVR of *Human-cl rhFVIII* – also over time.
- To investigate the immunogenic potential of *Human-cl rhFVIII* by inhibitor titre.
- To assess clinical efficacy of *Human-cl rhFVIII* in surgeries.
- To assess safety of *Human-cl rhFVIII* in terms of adverse event (AE) monitoring.

Outcomes/endpoints

The efficacy of prophylactic treatment on the basis of the frequency of spontaneous breakthrough BEs/months under prophylactic treatment was assessed as excellent, good, moderate or poor as described for GENA-08.

Efficacy assessments for the treatment of BEs and during surgery were the same as described for GENA-01 and GENA-08.

Sample size

No inferential analysis involving formal testing was planned in this uncontrolled study. Consequently, no formal sample size estimation was performed, but the sample size was chosen to satisfy current CHMP recommendations. According to the CHMP Guideline on the Clinical Investigation of new FVIII products, a study in children is to be performed in at least 50 patients

suffering from severe haemophilia A. Of those 50, at least 25 should be aged between 2 and 5 and at least 25 aged between 6 and 12 years. It was thus planned to include a total of 60 patients (30 aged 2–5 years and 30 aged 6–12 years) into the study in order to be able to compensate for discontinuations. 26 patients – 13 of each age cohort – were to participate in the PK phase (Phase I) of the study.

Randomisation

The study design did not require any randomisation. Study data were stratified as follows:

- Patients aged 2–5 years / with PK
- Patients aged 2–5 years / without PK
- Patients aged 6–12 years / with PK
- Patients aged 6–12 years / without PK.

Blinding (masking)

Not applicable.

Statistical methods

Statistical Study Conduct

A statistical analysis plan was compiled as a separate document. No inferential statistics, but rather exploratory and descriptive statistics were planned for this uncontrolled efficacy trial. The statistical analysis of the primary, secondary and safety endpoints was thought to be understood in the exploratory sense.

The final subject disposition, i.e. the identification of significant violations to consider for the PP, the PK-PP, and the PROPH-PP population and the assignment of each subject to these analysis populations, was to be the joint decision of the trial statistician and the responsible medical expert in a data review meeting prior to database lock.

Statistical analysis of the primary endpoints

All results were stratified by the age groups (2-<6 years, 6-12 years) and in total. Efficacy was evaluated by descriptive statistics. The frequency of BEs under prophylactic treatment was calculated after a total of 50 EDs and at the end of the study in order to assess overall efficacy. The frequency of spontaneous BEs only and for all BEs under prophylactic treatment was calculated. The frequencies of traumatic BEs and of all (spontaneous, traumatic, other) BEs were defined as secondary analyses. The efficacy of *Human-cl rhFVIII* in the treatment of breakthrough BEs and during surgical prophylaxis were assessed as described for GENA-08.

Results

Participant flow

See Figure 4.

Recruitment

This study was conducted at 15 investigational centres in the UK, Czech Republic, Poland, Russia, Turkey, France and Romania. The trial was initiated on 27 December 2010 and completed on 6 November 2012.

Conduct of the study

There were two amendments to the CSP dated 22-Mar-2010. Amendment 1, dated 17-Jun-2011, addressed the issue of prophylactic dosing. After it was realised that patients were reluctant to enter the study because of the every-other-day dosing specified in the protocol, the dosing recommendation was changed to also allow 3-times-weekly dosing.
Amendment 2, dated 07-Oct-2011, addressed the required number of evaluable patients for PK analysis. This was done in response to the change in EMA guideline specifying that 12, not 13, evaluable patients per age cohort need to undergo PK evaluation.

There were several changes in the planned analyses compared with the latest version of the protocol expected to have limited impact on the study results.

Major protocol deviations were recorded in 3 of the 59 patients. Two patients received other FVIII concentrate after the start of prophylaxis. One patient had an extremely long gap between 2 doses after start of prophylactic treatment. Fifty-seven patients had a least one minor protocol deviation (higher prophylactic dose, wash-out period for recovery was not respected; FVIII sampling was outside the specified time window).

Baseline data

All patients enrolled in the study were male. In accordance with the inclusion criteria, patients were between 2 and 12 years of age, with a median age of 6 years. Total HJHS at baseline (range 0 [best] to 148 [worst]) ranged from 0 to 11, with a median score of 0, indicating good joint health, as expected in this paediatric population of PTPs.

The main historical BE sites were the ankle (11.9% of patients both sides; 11.9% and 18.6% left and right ankle, respectively; 1.7% unknown), the knee (8.5% of patients both sides; 10.2% and 11.9% left and right knee, respectively) and the leg (10.2% of patients both sides; 5.1% and 3.4% left and right leg, respectively; 1.7% unknown).

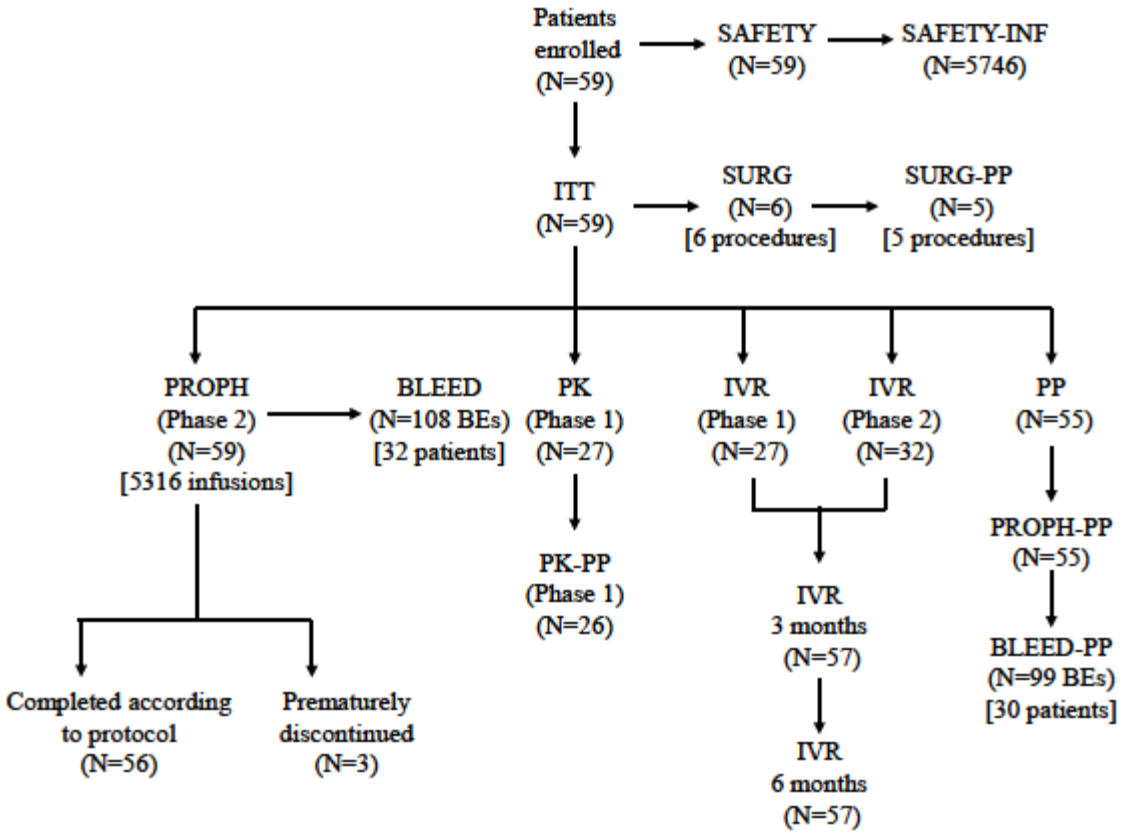
All patients were white and not of Hispanic or Latino ethnicity; most patients had blood type O (25; 42.4%) or A (24; 40.7%). Known FVIII gene defects were documented for 56 out of the 59 patients; most were single occurrence, except for intron 22 inversion and missense mutations, which were observed in 25 (42.4%) and 11 (18.6%) patients, respectively. A family history of haemophilia was documented in less than half of the patients (24; 40.7%); 4 patients (6.8%) had a family history of inhibitor activity.

In the 6 months prior to the study, 33 of the 59 patients had received rFVIII concentrates and 27 of the 59 patients had received pdFVIII concentrates; 5 of the 59 patients had received both pdFVIII and rFVIII concentrates in the previous 6 months. Six patients had previously received on-demand treatment and 53 patients had received FVIII concentrates as prophylaxis.

Numbers analysed

Patient disposition and patient numbers analysed are shown in Figure 4.

Figure 4: Patient disposition



BLEED = study population of BEs treated with *Human-cl rhFVIII*; PROPH = study population of patients receiving prophylaxis; ITT = intention-to-treat; IVR = in vivo recovery; PK = pharmacokinetics; PP = per-protocol; SAFETY = study population of patients in safety analysis; SAFETY-INF = all infusions within safety population; SURG = study population of surgeries treated with *Human-cl rhFVIII*.

A total of 59 patients were enrolled in this study. Of these, 56 completed the study and 3 prematurely discontinued. All 59 patients were included in the safety and the ITT populations. All 59 patients received prophylactic treatment with *Human-cl rhFVIII* (PROPH population). A total of 108 breakthroughs BEs were treated with *Human-cl rhFVIII* (BLEED population) in 32 patients in the PROPH population. Surgery with at least one dose of *Human-cl rhFVIII* was performed in 6 patients during 6 procedures.

Outcomes and estimation

Prophylaxis

All 59 patients received prophylactic treatment with *Human-cl rhFVIII* and were included in the PROPH population. The total number of prophylactic injections was 5316.
The average dose per infusion (38.9 IU/kg) was towards the higher end of 30–40 IU/kg range due to the rounding up of doses to the nearest vial. As only whole 500 IU vials were infused, in patients in whom, for example, one vial would be at the lower end of, or below, the recommended range, the investigators chose to increase the dose at some point to two vials and have a dose at the top end of the range.

EDs and dosage of *Human-cl rhFVIII* for prophylaxis are shown in Table 20.

Table 20: EDs and dosage for prophylactic treatment with *Human-cl rhFVIII* (PROPH population, N=59)

Parameter	Mean	SD	Median	Range
Number of EDs	89.8	22.33	87.0	19–149
Number of infusions per ED	1.0	0.02	1.0	1.0–1.1
Duration of prophylactic treatment, months	6.6	1.4	6.3	1.3–9.8
Total dose of *Human-cl rhFVIII*, IU	95,860	57,044	85,000	19,000–360,000
Average amount of *Human-cl rhFVIII* per month of study, IU/kg/month	527.7	112.3	521.9	332.3–888.5
Average dose of *Human-cl rhFVIII* per infusion, IU	1049	486	1000	300–3000
Average dose of *Human-cl rhFVIII* per infusion, IU/kg	38.9	7.2	37.8	26.0–56.7

ED = exposure day; IU = international units; PROPH = study population of patients receiving prophylaxis; SD = standard deviation.

A total of 129 BEs were experienced by 39 patients during the prophylactic treatment. Of these 129 BEs, 74 (57.4%) were traumatic, 45 (34.9%) were spontaneous and 10 (7.7%) were classified as “other”. In the PROPH population, 20 (33.9%) patients did not experience any BEs and 14 patients (23.7%) experienced only one BE during the study. Overall efficacy assessments of *Human-cl rhFVIII* prophylaxis are shown in Table 21.

Table 21: Overall efficacy assessment of prophylactic treatment at the end* of the study (PROPH population, N=59)

Overall assessment† (monthly BE rate)	Spontaneous BEs N (%)	Traumatic BEs N (%)	All BEs N (%)
Total, n=59			
Excellent	56 (94.9)	55 (93.2)	49 (83.1)
Good	1 (1.7)	3 (5.1)	5 (8.5)
Moderate	2 (3.4)	-	3 (5.1)
Poor	-	1 (1.7)	2 (3.4)
Age 2–5 years, n=29			
Excellent	28 (96.6)	29 (100.0)	27 (93.1)
Good	1 (3.4)	-	1 (3.4)
Moderate	-	-	1 (3.4)
Poor	-	-	-
Age 6–12 years, n=30			
Excellent	28 (93.3)	26 (86.7)	22 (73.3)
Good	-	3 (10.0)	4 (13.3)
Moderate	2 (6.7)	-	2 (6.7)
Poor	-	1 (3.3)	2 (6.7)

* Includes all BEs between start of prophylactic treatment and last prophylactic treatment + 2 days or completion visit, whichever came first. BEs between start of treatment for surgery and re-start of prophylactic treatment after surgery were excluded.
† Excellent = <0.75 BEs/month, Good = 0.75–1.0 BEs/month, Moderate = >1.0–1.5 BEs/month and poor = >1.5 BEs/month.
BE = bleeding episode; ED = exposure day; n = number of patients in analysis group; PROPH = study population of patients receiving prophylaxis.

The mean rate of spontaneous BEs was 0.123 BEs/month (median 0; range 0–1.13) at the end of the study; the monthly rate of spontaneous BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.089 vs. 0.156 BEs/month). The monthly rate of traumatic BEs was higher than that observed for spontaneous BEs, although rates in the two age groups followed a similar pattern. The mean rate of traumatic BEs was 0.192 BEs/month (median 0.129; range 0–1.53) at the end of the study; the monthly rate of traumatic BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.113 vs. 0.268 BEs/month). The mean rate of all BEs was 0.338 BEs/month (median 0.156; range 0–1.70) at the end of the study; the monthly rate of all BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.213 vs. 0.459 BEs/month).

Treatment of breakthrough BEs

A total of 108 BEs (32 patients) were treated with *Human-cl rhFVIII* and comprised the BLEED population.

Of the 108 BEs, 65 (60.2%) were traumatic, 36 (33.3%) were spontaneous and 7 (6.5%) were classified as "other". Sixty-one (56.5%) of the BEs were minor, 46 (42.6%) were moderate to major and one (0.9%) was of unknown severity. There were no major to life-threatening BEs.

The most frequent sites of BEs were the ankle (21 BEs) and the knee (15 BEs).

When one infusion was given to treat several BEs simultaneously, the number of EDs and the number of dosages were counted only once. A total of 216 infusions were used in this analysis. The median number of infusions to treat a BE across all severities was 1.0 and the mean number was 2.1 (range 1–22). The difference between the mean and median values is mainly due to 1 BE that was treated with 22 infusions.

EDs and dosage of *Human-cl rhFVIII* for the treatment of BEs are shown in Table 22. For the whole BLEED population, the mean dose of *Human-cl rhFVIII* per infusion was 45.1±12.61 IU/kg (range 25–88 IU/kg) across all severities; it was 43.9±12.17 IU/kg for minor BEs and 45.7±12.84 IU/kg for moderate to major BEs. The median total dose used for the treatment of a BE was 1,612.5 IU (range 500–33,000).

Table 22: EDs and *Human-cl rhFVIII* infusions for treatment of BEs (BLEED population, 108* BEs [216 Infusions] in 32 patients)

Parameter	Mean	SD	Median	Range
Number of EDs (for BEs)	1.9	1.85	1	1–10
Dose of *Human-cl rhFVIII* per BE, IU	2528	3716.2	1612.5	500–33,000
Dose of *Human-cl rhFVIII* per BE, IU/kg	95.9	169.3	43.9	25–1521
Number of infusions per BE	2.1	2.95	1	1–22
Dose of *Human-cl rhFVIII* per infusion, IU	1189	450.1	1000	500–3000
Dose of *Human-cl rhFVIII* per infusion, IU/kg	45.1	12.61	40.0	25–88

* Several simultaneous BEs (patient 030103, BE: 2,3; patient 035104, BE: 1,2; patient 036501, BE: 1,2,3; patient 037401, BE: 2, 4, 5) are counted only once in this analysis, because the infusions for these BEs were given to treat several bleedings simultaneously. Thus, the number of EDs and the number of dosages are counted only once; thus number of infusions for this analysis was 216.
BE = bleeding episode; BLEED = study population of BEs treated with *Human-cl rhFVIII*;
ED = exposure day; IU = international units; SD = standard deviation.

During the study, 68.6% BEs were treated with one infusion and 81.3% with one or 2 infusions. Six (5.9%) BEs required 3 infusions and 4 (3.9%) BEs required 4 infusions. Two BEs each (2.0% each) required 5, 6 and 8 infusions and 1 BE each (1.0% each) required 12, 15 and 22 infusions.

Efficacy assessments for the treatment of BEs are shown in Table 23.

Table 23: Efficacy assessment for treatment of BEs according to severity (BLEED population, 108 BEs in 32 patients)

Severity of BE* Efficacy rating	Number of BEs					
	Overall		Aged 2–5		Aged 6–12	
	N	%	N	%	N	%
Any	n=108		n=33		n=75	
Excellent	77	71.3	21	63.6	56	74.7
Good	12	11.1	6	18.2	6	8.0
Moderate	17	15.7	6	18.2	11	14.7
None	2	1.9	0	0	2	2.7
Minor	n=61		n=20		n=41	
Excellent	53	86.9	15	75.0	38	92.7
Good	7	11.5	5	25.0	2	4.9
Moderate	1	1.6	0	0	1	2.4
None	0	0	0	0	0	0
Moderate to major	n=46		n=13		n=33	
Excellent	23	50.0	6	46.2	17	51.5
Good	5	10.9	1	7.7	4	12.1
Moderate	16	34.8	6	46.2	10	30.3
None	2	4.3	0	0	2	6.1

The severity of 1 BE (patient 034104, 6–12 years subgroup) was classed as unknown and had an efficacy rating of excellent.
BE = bleeding episode; BLEED = study population of BEs treated with *Human-cl rhFVIII*; n = number analysed.

The 2 BEs for which *Human-cl rhFVIII* efficacy was rated as “none” were of moderate to major severity and are described in more detail:

One patient experienced a spontaneous moderate to major bleeding in the right ankle that was treated with 12,000 IU of *Human-cl rhFVIII* over 10 EDs (12 infusions) -subsequently withdrawn from the study, after 71 days in the study, due to therapy failure. The second patient experienced a spontaneous moderate to major BE in the right arm that was treated with a total of 5,000 IU of *Human-cl rhFVIII*. He received 47.6 IU/kg (1000 IU) *Human-cl rhFVIII* daily over 5 EDs, with no dose changes and no treatment with other FVIII concentrates. He was treated with Efferalgan and Doliprane (paracetamol) during the course of this BE. He experienced 8 other BEs, the treatment of which was rated as excellent or good in 5 and moderate in 3 BEs and the overall efficacy of prophylactic treatment for this patient was rated as moderate. This patient’s IVR values ranged from 1.24 to 1.59%/IU/kg.

The IDMC adjudicated on all personal efficacy assessments for the treatment of BEs, primarily considering the number of infusions administered for each BE. In three cases the IDMC assessment differed from the personal assessment. In one case (patient 030202, BE no. 4) in which the personal assessment was rated as "good", efficacy was judged "moderate" by the IDMC; in another case (BE no. 4) in which the personal assessment had been "excellent", efficacy was rated as "good" by the IDMC. In addition, in another case (BE no. 1) in which the personal assessment was rated as "moderate", efficacy was judged as "none" by the IDMC during the final IDMC meeting for this study after database lock.

Surgery

Six patients underwent 6 planned surgical procedures under *Human-cl rhFVIII* treatment (Table 24). All surgeries were major.

Table 24: Description and efficacy rating of surgical procedures (SURG population, 6 surgeries in 6 patients)

Patient	Description of surgery	Infusions (N)	EDs (N)	Total dose IU/kg	Overall efficacy rating
034114	Port catheter implantation	20	7	593.22	Excellent
036102	Circumcision	5	3	183.33	Excellent
036103	Port catheter replacement	3	2	150.00	Excellent
036301	Port catheter implantation	5	4	233.33	Excellent
036302	Port catheter implantation	4	3	170.00	Excellent
036303	Port catheter implantation	4	2	160.00	Not assessed*

* This patient was later diagnosed with VWD and withdrawn from the study.
ED = exposure day; IU = international units; SURG = study population of surgeries treated with *Human-cl rhFVIII*; VWD = von Willebrand disease.

Excluding the patient with VWD, individual doses for the 5 surgeries ranged from 28.25 IU/kg to 56.50 IU/kg. All 5 patients received a 50 IU/kg loading dose before their surgeries, except patient 034114 who received 56.50 IU/kg. No maintenance doses during surgery were required for any patient. The number of infusions ranged from 3 to 5 and the number of EDs ranged from 2 to 4, with the exception of one patient who underwent a port catheter implantation and received 20 infusions during 7 EDs. Blood loss during all these surgeries was minimal (2–10 mL), with the highest amount of 10 mL each for a port catheter implantation and a circumcision. One port catheter implantation surgery had higher than average expected blood loss; however, the actual blood loss was very low (4 mL) and not higher than the maximum expected blood loss (5 mL). Efficacy was rated as excellent by both the surgeon and the haematologist for all 5 of these surgeries. One patient was treated with tranexamic acid during the hospitalisation after surgery. In the patient with VWD, a total dose of 160 IU/kg (4 infusions of 40 IU/kg) was administered for

a port catheter replacement. Actual blood loss (5 mL) was the same as the maximum expected volume. Efficacy was not assessed in the patient. The mean duration of surgeries was 33.3±6.06 minutes, ranging from 30 to 45 minutes. The mean expected duration was 40 minutes for all surgeries.

Table 25: Summary of EDs and dosages administered for surgeries (SURG population, 6 surgeries in 6 patients)

Parameter	Mean	SD	Median	Range
Total dose of *Human-cl rhFVIII*, IU	5816.7	2619.5	5000	3400–10,500
Total dose of *Human-cl rhFVIII*, IU/kg	248.31	171.47	176.67	150–233.3
Pre-operative loading dose, IU/kg	49.42	5.30	50	40–56.5
Infusions after end of surgery, IU/kg	198.90	167.87	126.67	120–536.7
Total dose of *Human-cl rhFVIII* per ED, IU/kg	69.31	12.10	68.06	56.7–84.7
Dose of *Human-cl rhFVIII* per infusion, IU	1062.50	359.08	1050	850–1500
Dose of *Human-cl rhFVIII* per infusion, IU/kg	40.92	7.26	41.25	29.7–50

ED = exposure day; IU = international units; SD = standard deviation; SURG = study population of surgeries treated with *Human-cl rhFVIII*.

Summary of main efficacy results

Prophylactic efficacy

The mean rate of all BEs was 0.338 BEs/month (median 0.156; range 0–1.70) at the end of the study; the monthly rate of all BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.213 vs. 0.459 BEs/month).

Haemostatic response

Efficacy assessments were available for all 108 BEs treated with *Human-cl rhFVIII*, 61 of which were minor, 46 were moderate to major and the severity of 1 BE was unknown. Efficacy was rated as excellent or good for 89 BEs (82.4%; excellent 71.3%, good 11.1%); 17 (15.7%) BEs had an efficacy rating of moderate and 2 (1.9%) BEs had an efficacy rating of “none”.

Numbers of infusions needed to stop a BE

During the study, 68.6% BEs were treated with one infusion and 81.3% with one or 2 infusions. Six (5.9%) BEs required 3 infusions and 4 (3.9%) BEs required 4 infusions. Two BEs each (2.0% each) required 5, 6 and 8 infusions and 1 BE each (1.0% each) required 12, 15 and 22 infusions.

FVIII consumption

For prophylaxis, the average dose per infusion was 38.9 IU/kg. The average amount of *Human-cl rhFVIII* per month was 527.7 IU/kg.

For the treatment of BEs, the mean dose of *Human-cl rhFVIII* per infusion was 45.1±12.61 IU/kg (range 25–88 IU/kg) across all severities; it was 43.9±12.17 IU/kg for minor BEs and 45.7±12.84 IU/kg for moderate to major BEs. The median total dose used for the treatment of a BE was 1612.5 IU (range 500–33,000).

Haemostatic response surgery

Six patients underwent 6 planned surgical procedures under *Human-cl rhFVIII* treatment. All surgeries were major. Excluding one patient who was subsequently diagnosed with VWD and for whom efficacy was not assessed, efficacy was rated as excellent by both the surgeon and the haematologist for all 5 major surgeries.

Ancillary analyses

Not applicable.

Summary of main studies

Table 26: Summary of efficacy for trial GENA-03

Title: Prospective clinical study in children with severe haemophilia A to investigate clinical efficacy, immunogenicity, PK, and safety of *Human-cl rhFVIII*		
Study identifier	GENA-03	
Design	Prospective, non-controlled, open label, multinational, multicentre phase III study	
	Duration of Phase I: Duration of Phase II Duration of III:	PK assessment (Cycles 1, 2) Recovery assessments Efficacy Phase
Hypothesis	Exploratory study to assess clinical efficacy of *Human-cl rhFVIII* in terms of prevention and treatment of (breakthrough) BEs in PTPs.	

Treatments groups	PK-PP population		Patients who underwent PK analysis with their previous FVIII concentrate and *Human-cl rhFVIII*. PK/IVR phases: 50 IU FVIII/kg BW (based on the labelled potency) for at least 50 EDs and at least 6 months. (N=27)
	PROPH population		Patients suffering from severe haemophilia A, who initiated prophylactic treatment with *Human-cl rhFVIII*. Efficacy phase: Every other day or 3 times weekly. The recommended dosage regimen was 30–40 IU FVIII/kg BW. Two dose escalations of each +5 IU FVIII/kg BW were allowed in case of an inadequate response (≥2 spontaneous BEs within one month) (N=59)
	BLEED population		Patients who experienced 108 BEs (36 spontaneous, 65 traumatic and 7 "other") that were treated with *Human-cl rhFVIII* (N=32, 108 BEs)
	SURG population		Patients who underwent surgical procedures during which *Human-cl rhFVIII* was used (N=6)
Endpoints and definitions	Primary endpoint	Clinical efficacy	Prophylaxis and treatment of breakthrough BEs
	Secondary Endpoints	PK parameters	$AUCT_{1/2}$, IVR, C_{max}, T_{max}, MRT, V_{ss} and CL.
		Incremental IVR	Over time
		Immunogenicity	Assessment of inhibitor titre
		Efficacy in surgeries	Overall efficacy assessment
		Safety	Monitoring of AEs
Database lock	06 November 2012		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population	ITT		

Efficacy of prophylaxis (N=59)	<u>BE type during prophylaxis:</u> Spontaneous (34.9%), traumatic (57.4%), other (10%) <u>Efficacy assessment of prophylaxis based on monthly spontaneous BE rate:</u> Excellent (94.9%), good (1.7%) [96.6% excellent or good], moderate (3.4%) <u>Efficacy assessment of prophylaxis based on monthly traumatic BE rate:</u> Excellent (93.2%), good (5.1%) [98.3% excellent or good], poor (1.7%) <u>Efficacy assessment of prophylaxis based on monthly all BE rate:</u> Excellent (83.1%), good (8.5%) [91.5% excellent or good], moderate (5.1%), poor (3.4%)
Efficacy of the treatment of BEs (N=32, 108 BEs)	<u>Severity of treated BE:</u> Minor (56.5%), moderate to major (42.6%), other (0.9%) <u>Type of treated BE:</u> Spontaneous (33.3%), traumatic (60.2%), other (6.5%) <u>Efficacy assessment for the treatment of BEs:</u> Excellent (71.3%), good (11.1%) [82.4% excellent or good], moderate (15.7%), none (1.9%)
Efficacy of surgical prophylaxis (N=6)	<u>Efficacy assessment (5 evaluable):</u> <u>Excellent (100%)</u>
Analysis description	**Primary Analysis:**
	No inferential analysis involving formal testing was planned in this uncontrolled trial. The sample size was determined by a CHMP guideline. Consequently, no formal sample size estimation was performed. The statistical analyses of the primary and secondary endpoints were descriptive.

AE = adverse event; AUC = area under the curve; BE = bleeding episode; BW = body weight; CHMP = Committee for Medicinal Products for Human Use; C_{max} = maximum plasma concentration; ED = exposure day; FVIII:C = FVIII coagulant activity; ITT = intention-to-treat; IVR = *in vivo* recovery; MRT = mean residence time; PP = per-protocol; PTP = previously treated patient; $T_{1/2}$ = half-life; T_{max} = time to maximum plasma concentration; V_{ss} = volume of distribution at steady state.

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

Efficacy data for *Human-cl rhFVIII* across pivotal studies are shown below for prophylaxis (Table 27), treatment of BEs (Table 28) and surgical prophylaxis (Table 29).

Table 27: Efficacy of prophylaxis across pivotal studies

	GENA-08	GENA-03
Spontaneous BEs per month	0.095 0–0.71	0.123 0–1.13
Traumatic BEs per month	0.082 0–0.68	0.192 0–1.53
All BEs per month	0.188 0–1.21	0.338 0–1.70

Data are mean, range.
BE = bleeding episode.

Table 28: Efficacy of treatment for BEs across pivotal studies

Severity of BE Efficacy rating	GENA-01	GENA-08	GENA-03
Any (N)	986§	28†	108‡
Excellent	60.3	71.4	71.3
Good	34.1	28.6	11.1
Moderate	5.5	–	15.7
None	–	–	1.9
Minor (N)	416	14	61
Excellent	75.0	85.7	86.9
Good	23.6	14.3	11.5
Moderate	1.4	–	1.6
None	–	–	–
Moderate to major (N)	566	14	46
Excellent	50.0	57.1	50.0
Good	41.7	42.9	10.9
Moderate	8.3	–	34.8
None	–	–	4.3
Major to life threatening (N)	3	–	–
Excellent	66.7	–	–
Good	33.3	–	–
Moderate	–	–	–
None		–	–

Data are percentages.
† For 2 BEs, no efficacy assessments were available.
‡ The severity of 1 BE was classed as unknown and had an efficacy rating of excellent.
§ Efficacy rating was missing for 1 BE.
BE = bleeding episode; N = number of BEs.

Table 29: Efficacy assessment for surgical prophylaxis across pivotal studies

Classification of surgery Efficacy rating	GENA-01	GENA-08	GENA-03
Any (N)	2	5	5†
Excellent	2	4	5
Good	–	–	–
Moderate	–	1	–
None	–	–	–
Minor (N)	1	1	0
Excellent	1	1	–
Good	–	–	–
Moderate	–	–	–
None	–	–	–
Major (N)	1	4	5†
Excellent	1	3	5
Good	–	–	–
Moderate	–	1	–
None	–	–	–

† Efficacy was not assessed in 1 additional patient who underwent port catheter implantation surgery due to the diagnosis of VWD in this patient.
N = number of surgeries.

Clinical studies in special populations

See Study GENA-03 in paediatric patients.

Supportive studies

Two studies (GENA-09 and its extension GENA-04) were conducted in a single centre in Russia. This patient population is characterised by less intensive haemophilia control and advanced sequelae e.g. target joints and a history of high rates of BEs with a high incidence of severe BEs.

Prophylactic efficacy in study GENA-09 was lowest of all studies (90.9% excellent or good). Still, even in this study, there was only 1 patient with poor prophylactic efficacy and only 2 with moderate efficacy. Importantly, at the end of GENA-04, the extension of study GENA-09, efficacy ratings markedly improved to excellent in all but one patient (94.4%), suggesting that long-term prophylactic treatment with *Human-cl rhFVIII* may improve outcomes even in patients who have previously received inadequate treatment.

Long-term prophylactic treatment (GENA-04) with *Human-cl rhFVIII* with an average dose of 34.6 IU/kg resulted in a decrease in both HJHS (total and gait) and BE rates compared with the patients' values prior to *Human-cl rhFVIII* prophylaxis. The overall efficacy of the prophylactic treatment was deemed excellent in 94% and good in 6% of the patients. With the average dose of 34.63 IU/kg per infusion for the treatment of BEs, the haemostatic efficacy rating of *Human-cl rhFVIII* was excellent or good for 83.7% of BEs.

Overall surgical efficacy was rated as excellent in 14 minor surgical procedures in GENA-09 and as excellent for 4 minor surgical procedures and good for 3 major surgeries in GENA-04 by the surgeon and the haematologist.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The design of the submitted pivotal clinical trials investigating the efficacy (GENA-01, GENA-08 and GENA-03) of *Human-cl rhFVIII* follows the requirements of the guideline for rFVIII products (EMA/CHMP/BPWP/144533/2009). One trial (GENA-01) investigated the efficacy of *Human-cl rhFVIII* for the on-demand treatment of BEs in 22 patients with severe haemophilia A. Two trials investigated the efficacy of *Human-cl rhFVIII* for the prophylaxis and treatment of breakthrough BEs in 32 adults (GENA-08) and 59 children (GENA-03). In all three studies, the investigation of the efficacy of *Human-cl rhFVIII* for surgical interventions was foreseen.

The included patient population of the three pivotal studies was multinational and comprised previously treated children (2-<12 years of age), 3 adolescents (12, 12 and 14 years) and adults suffering from severe haemophilia A, which was defined as FVIII levels <1% for the paediatric trial and ≤1% for the adult/adolescent trials. According to the current guideline, severe haemophilia patients with FVIII activity <1% should be enrolled. According to the guideline CPMP/BPWG/1561/99 rev.1 valid at the time of the initiation of the trials in the adolescent and adult population, patients with FVIII activity ≤1 were the target population. Therefore the definition of severe haemophilia A is considered acceptable.

The primary efficacy analysis for GENA-01 (on-demand treatment) focused on the proportion of BEs with successful treatment (rated as “good” or “excellent” in the efficacy assessment at the end of the BE). A BE was considered as treatment failure, if the efficacy assessment was “moderate” or “none” or if another FVIII product was used.

For GENA-08 (prophylaxis in adults), the overall efficacy assessment after a total of 50 EDs and after end of the study was based upon the frequency of spontaneous BEs (i.e. traumatic, post-operative or “other” BE not included). The frequency of traumatic BEs and all (spontaneous, traumatic, other) BEs were addressed, in addition.

For GENA-03 (prophylaxis in children), two calculations for the primary endpoint on BE rate during prophylaxis were specified (frequency of spontaneous BEs only and all BEs). The efficacy of *Human-cl rhFVIII* in on-demand treatment of breakthrough BEs was assessed by a four-point efficacy assessment at the end of a BE. Additionally, the proportion of BEs successfully treated with *Human-cl rhFVIII* was evaluated for all BEs. “Successfully treated” were all “excellent” and “good” efficacy ratings of treated BEs.

For all surgical procedures, efficacy was to be evaluated by the surgeon and the haematologist at the end of the surgery and on the last post-operative day. In addition to the four-point scale (excellent, good, moderate, none), the proportion of surgeries with successful treatment according to the overall efficacy assessment was calculated. “Successfully treated” was defined as all “excellent” and “good” efficacy ratings of surgical procedures.

Generally, dosing recommendations for prophylaxis and the treatment of BEs were in line with the World Federation of Hemophilia (WFH) Guideline recommendations as well as with the core SmPC. A higher consumption for prophylaxis per month was observed in the paediatric study (median: 521.9 IU/kg) as compared to prophylaxis for the adults (468.7 IU/kg/month).

The protocol violations noted in each study were examined in a routine GCP inspection of trials GENA-01 and GENA-03 initiated by the EMA. The inspectors concluded that the data in the Clinical Study Report presented as a whole, was considered reliable and suitable for assessment in an MAA.

In general, the clinical study programme follows the current Clinical Guideline regarding objectives, duration and outline. Prophylaxis, on-demand treatment and prophylaxis in surgical procedures have been documented and evaluated.

Patient selection, in general, follows widely accepted use in comparable study-designs.

Providing data from a differing patient population from Russia (GENA-09 and GENA-04) is considered to be supportive, only. However, these data are valuable with respect to efficacy of FVIII substitution in a specific and challenging patient population with advanced disease sequelae representing treatment conditions less developed than in Western European countries.

According to long-standing experience with similar products, the usual doses are 20 to 40 IU of FVIII per kg body weight (IU/kg BW) at intervals of 2 to 3 days for prophylaxis against BEs in patients with severe haemophilia A. In some cases, especially in younger patients, shorter dosage intervals or higher doses may be necessary. Presented dosages are within this range, although at the upper margins. These recommendations are included in the SmPC Section 4.2.

The dosing interval has been chosen as “every other day” which is in line with recent positive experience with short dosing intervals with respect to overall FVIII consumption. Justification of such an interval to maintain stable FVIII concentrations above 1% activity – without reaching high peak levels, is acknowledged.

The suggested assessment for efficacy in prophylaxis based upon monthly BE rates is considered to be acceptable. Furthermore, annual (monthly) consumption per kg BW (bodyweight) in prophylaxis is requested by the Clinical Guideline has been provided (see below).

Recommended dosing for BEs and surgeries was in line with recommendations for clinical studies and the core SmPC. It is considered to be acceptable to allow individual dosage according to the clinical situation.

An evaluation score for haemostatic efficacy has been used in addition to the number of infusions and the time for resolution after a BE, which is accepted. FVIII dosage per kg and BE has been presented, as well as differentiation of all evaluations for “true” on-demand BEs versus BEs under prophylaxis (breakthrough BEs).

Evaluation of efficacy in surgery has been based upon objective criteria. Furthermore, data on blood loss, need for transfusions and FVIII consumption per kg and surgical procedure have been provided in accordance with the Clinical Guideline.

Patient numbers in general meet the recommendations of the Guideline. A high number of protocol violations in each study was investigated in a routine GCP inspection of trials GENA-01 and GENA-03, which had a positive outcome.

Efficacy data and additional analyses

In GENA-01, 94.4% (931/986) of the BEs were treated with excellent or good efficacy (60.3% excellent, 34.1% good). A large majority of BEs required only one *Human-cl rhFVIII* infusion (841/986, 91.4%) and 53 BEs (5.8%) required 2 infusions. The mean BE rates per patient during the prophylactic treatment period at the end of GENA-08 study were 0.188/month (median 0.074; range 0–1.21) for all types of BEs. Personal efficacy assessments were available for 28 BEs. Efficacy ratings on a four-point scale were excellent or good for all BEs (71.4% (20/28) excellent, 28.6% (8/28) good).

Due to the small patient population uncertainty on the estimates is large. The applicant provided analyses across trials as an obvious means to increase efficiency and the precision of estimates, both for prophylactic efficacy and for haemostatic success in treatment of BEs with *Human-cl rhFVIII*, and for the surgical procedure setting.

Efficacy of prophylaxis: For prophylaxis, no specific guidance regarding efficacy evaluation is available through the Clinical Guideline. However, the suggested recording of monthly BE rates represents an instrument for describing efficacy in the respective regimen. Interpretation of the BE rates might well be influenced by age (children) or predisposition (target joints). From the presented data, differentiation of spontaneous, traumatic and overall BEs is considered to be of relevance.

An overview on efficacy assessment based on monthly BE rates (spontaneous, traumatic and all BEs) has been provided.

Efficacy of treatment of BEs: An individually driven 4-point scale is suggested by the Clinical Guideline, which has been met by the study design. From the study description, it might be assumed, that GENA-01 represents mostly “true” on-demand BEs whereas the BEs from other studies represent breakthrough BEs.

Further variability between the studies may mainly be attributed to the differing patient population. However, the significant differences e.g. between GENA-09 and GENA-08 demonstrate that efficacy assessment in this kind of condition remains highly challenging.

According to the Clinical Guideline, dose per kg (IU/kg) per BE should be provided. Median dose per kg and BE was 30.9 IU/kg (range 8–657) in study GENA-01. For completeness, overall mean dose/kg per BE and mean dose/kg per major versus moderate versus minor BE have been provided.

Monthly consumption of FVIII for prophylaxis in GENA-08 (468.7 IU/kg) and GENA-03 (521.9 IU/kg) has been provided and is reflected in the SmPC.

Consumption data in studies GENA-09 and GENA-04 are considered supportive, as these studies represent a specific patient population. Improvement of BE control and even joint health is noted.

Efficacy of surgical prophylaxis: Data illustrate variability of dosage for minor vs. major surgical procedures and the differences of standards between Western European countries and e.g. Russia: Total doses as well as supportive measures differ substantially, but are considered to represent known variability.

Taking all presented studies together, data regarding surgical procedures meet the requirements of the Clinical Guideline of a minimum of 10 major surgical procedures in at least 5 patients.

Assessment of paediatric data on clinical efficacy

Paediatric patients were addressed in study GENA-03. Patient characteristics were well described. There were only 3 patients in the paediatric age-group between 12 and 18 years (ages 12, 12 and 14 years), with a gap between the ages of 14 to 18 years. Dosing in paediatric patients will remain challenging due to specific needs in these age groups. Children of less than 2 years of age have not been included in the clinical trial. However, in the context of this congenital disease, there is a potential relevant use in children below 2 years. Taking into account the current data as well as the fact that it is a recombinant product, the knowledge with existing therapies could support the use of the product in this age group. As there is an absence of any concern (safety concern, posology or immunological aspect) that could impose a restriction, it was considered that the data can be extrapolated so this age range is covered by the indication, provided the patients are previously treated.

The mean rate of all BEs in GENA-03 (prophylaxis) was 0.338 BEs/month (median 0.156; range 0–1.70) at the end of the study; the monthly rate of all BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.213 vs. 0.459 BEs/month). During the study, 68.6% of breakthrough BEs were treated with one infusion and 81.3% with one or 2 infusions.

Differences in the monthly BE rate and dosage between paediatric and adult patients support the phenomenon of children requiring higher doses and shorter intervals of dosage for sufficient treatment.

Analysis of BE frequency as an efficacy assessment in prophylaxis for two age-subgroups shows higher frequency for the older (6–12 years) than for the younger (2–5 years) age-group. Consumption was 519 IU/kg monthly or 6230 IU/kg per year, which has been reflected within the SmPC.

The number of infusions for treatment of a breakthrough BE was between 1 and 22 with the majority being 1 or 2 infusions. However, BEs requiring 5, 6, 8, and even 12, 15 and 22 infusions were recorded. Median dose per kg and BE was 43.9 IU/kg (range 25-1521).

Statistical analyses using methods compatible with the data type of BE rates with appropriate confidence intervals have been provided. Additional analyses were provided aiming to estimate the success of treating BEs in a manner which accounts for this situation or which is not prone to the correlated structure of the data. Furthermore, due to the small patient population analyses were performed across trials for prophylactic efficacy, for haemostatic success in treatment of BEs (in the on-demand and the prophylaxis setting), and for the surgical procedure setting which confirm the individual findings.

2.5.4. Conclusions on the clinical efficacy

Efficacy has been analysed for prophylaxis, on-demand treatment, treatment of breakthrough BEs, and prophylaxis for surgical procedures. Study designs, selection and number of patients, assessment tools and results are in general adequate for supporting efficacy of Nuwiq. Minor additional changes or supplements to the SmPC were requested to adequately reflecting the efficacy profile.

The available clinical data for Nuwiq suggest that it could be an efficacious new FVIII product for the prevention and treatment of BEs in PTPs with haemophilia A. Uncertainties concerning the multitude of protocol deviations were clarified by GCP inspections and adequately addressed by the applicant.

2.6. Clinical safety

The clinical safety of *Human-cl rhFVIII* was assessed in 135 patients with severe haemophilia A in 5 studies with 59 patients under the age of 12 years. All clinical safety data were adjudicated by the IDMC. To date, there have been no reports of virus transmission, hypersensitivity, thromboembolism or inhibitor development related to *Human-cl rhFVIII*.

Patient exposure

135 individual patients received a mean of 104,813 to 585,489 IU (1835 to 6289 IU/kg) of *Human-cl rhFVIII* by a mean of 54.9 to 228 infusions over a mean of 53.3 to 226 EDs and over a mean period of 179.9 to 455.6 days during the clinical studies. Across the 5 studies, patients received a total of 32,650,787 IU (549,033 IU/kg) of *Human-cl rhFVIII* by 16,134 infusions over 15,950 EDs (Table 30).

Table 30: Study drug exposure

Study drug exposure (SAFETY populations)					
Parameter	GENA-01	GENA-08	GENA-03	GENA-09	GENA-04*
Number of patients	22	32	59	22	18
Number of EDs/patient	53.3 18–97	90.3 17–105	96.1 24–152	97.7 79–132	226 14–299
Number of infusions/patient	54.9 18–115	91.3 17–113	97.4 26–152	97.9 79–132	228 14–319
Total dose, IU	135,947 68,395–	248,516 61,545–	104,813 24,005–	226,576 143,500–	585,489 34,000–

	279,150	389,728	374,225	371,250	996,550
Total dose, IU/kg	1835 768–3443	3062 555–3949	3829 1050–7180	3253 2670–4474	6289 4825–8629
Duration of study (days)	342.7 205–674	179.9 39–218	208.6 49–338	201.8 191–241	455.6 33–563
Data are mean, range. *The 18 patients in GENA-04 also participated in GENA-09. ED = exposure day; IU = international units. .					

Adverse events

Potential safety concerns associated with the use of rFVIII concentrates include allergic-type hypersensitivity reactions and the possibility of formation of neutralising antibodies (inhibitors) to FVIII. The risk of transmission of infective agents with rFVIII is virtually completely eliminated. Three aspects of the safety of *Human-cl rhFVIII* were evaluated: tolerability, immunogenic potential and safety laboratory tests.

AEs (272) were reported in 79 patients. All of these events occurred in patients under treatment with *Human-cl rhFVIII*. The most commonly affected system organ class (SOC) was that of infections and infestations, which were recorded for 57 patients.

Twelve of the 242 AEs in 9 patients were classified as serious. Eight AEs were considered to be possibly or probably related to *Human-cl rhFVIII* treatment (Table 31).

Table 31: AEs possibly / probably related to treatment

Study, centre/ patient	MedDRA preferred term	Intensity	Outcome	Causality
GENA-08				
03/080301	Injection site pain	Mild	Recovered/Resolved	Possible
04/080402	Vertigo	Mild	Recovered/Resolved	Possible
	Dry mouth	Mild	Recovered/Resolved	Possible
	Paraesthesia	Mild	Recovered/Resolved	Possible
	Injection site inflammation	Mild	Recovered/Resolved	Possible
GENA-03				
21/032102	Back pain	Mild	Recovered/Resolved	Possible
63/036301	Headache	Mild	Recovered/Resolved	Possible
GENA-04				
01/040109	Anti-FVIII antibody positive	Mild	Unknown	Probable

AE =adverse event; FVIII = coagulation factor VIII; MedDRA = medical dictionary for regulatory activities.

Serious adverse event / deaths / other significant events

One death occurred in the GENA-08 study that was deemed not related to *Human-cl rhFVIII* administration. This patient had been diagnosed with epilepsy and had been taking an antiepileptic (oxycarbazepin, 300 mg twice a day) since 1997. The last documented IMP infusion according to the patient diary was 48 days before his death following a *status epilepticus*. The case record form (CRF) states acute respiratory and cardiovascular failure as the cause of death.

There were 11 additional serious AEs (SAEs) in 8 patients in the 5 studies: 3 SAEs (in 2 patients) in GENA-01, 7 SAEs (in 5 patients) in GENA-03 and 1 SAE (in 1 patient) in GENA-08. An overview is presented in Table 32.

Table 32: Listing of SAEs

Study, centre/ patient	MedDRA preferred term	Reason for seriousness	Outcome	Causality
GENA-01				
010101	Depression suicidal	Is life-threatening Requires/prolongs hospitalisation	Recovered, resolved	Not related
010202	Hepatic encephalopathy	Requires/prolongs hospitalisation Other important medical event	Recovered, resolved	Not related
	Hepatic cirrhosis	Requires/prolongs hospitalisation Other important medical event	Not recovered, Not resolved	Not related
GENA-08				
081103	Traumatic fracture	Required hospitalisation	Recovered/Resolved	Not related
GENA-03				
030104	Device-related infection	Required hospitalisation	Recovered/Resolved	Not related
034114	Head injury	Required hospitalisation	Recovered/Resolved	Not related
034117	Head injury	Required hospitalisation	Recovered/Resolved	Not related
036302	Acute tonsillitis	Required hospitalisation	Recovered/Resolved	Not related
	Upper respiratory tract infection	Required hospitalisation	Recovered/Resolved	Not related
	Lower respiratory tract infection	Required hospitalisation	Recovered/Resolved	Not related
036501	Haemarthrosis	Required hospitalisation	Recovered/Resolved	Not related

MedDRA = medical dictionary for regulatory activities; SAE = serious adverse event.

Laboratory findings

Routine laboratory tests for haematology parameters, clinical chemistry and electrolytes were performed in all studies. Urinalysis was additionally performed in studies GENA-01 and GENA-09. If abnormal values of ALT or AST persisted for more than one week, viral serology (hepatitis B virus [HBV], hepatitis C virus [HCV]) and PCR testing was to be performed to rule out HBV and HCV infection. In addition, PCR testing of the corresponding IMP batches was to be performed. No patients have been found to be positive for these viruses in any studies so far.

Safety in special populations

No specific evaluation regarding paediatric safety has been provided. However, a comparative AE listing reflecting AEs by SOC and preferred term (PT) was provided showing accumulations for

AEs typical for the paediatric age-group, such as upper respiratory infections or injuries. No evidence for additional age-specific signals can be identified according to this information.

Safety related to drug-drug interactions and other interactions

Not applicable.

Discontinuation due to adverse events

One patient in the GENA-01 study terminated the study prematurely due to loss to follow-up. Two patients each did not complete the GENA-04 and GENA-08 studies. Three of these 4 patients discontinued prematurely due to withdrawal of consent after 14, 17 and 117 EDs; in GENA-08 one patient died after 67 EDs following a status epilepticus that was deemed unrelated to study treatment. Two patients were withdrawn in the GENA-03 study. One patient was withdrawn due to protocol violation (a new diagnosis of VWD and thus a violation of the inclusion criteria after 24 EDs) and a second patient was withdrawn due to therapy failure after 42 EDs. There were no premature discontinuations in the GENA-09 study.

Post-marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

135 individual patients were treated with *Human-cl rhFVIII* in 5 completed clinical studies (GENA-01, GENA-08, GENA-03, GENA-09 and GENA-04) and provided safety data that were adjudicated by the IDMC.

Dosages with respect to PK, treatment for BEs, prophylaxis in surgery and prophylaxis regimen have been provided and are considered to range in the upper margins of common use of similar medicinal products.

272 AEs were reported in 79 patients. All of these events occurred in patients under treatment with *Human-cl rhFVIII*. Twelve of the 242 AEs in 9 patients were classified as serious and eight AEs were considered to be possibly or probably related to the treatment.

Description of common AEs is considered to reflect a profile of similar products. Outcome of the very low titre anti-FVIII antibody as measured by ELISA (inhibitor titre <0.6 BU) from GENA-04 reflected as “unknown” was further discussed and found to be non-neutralising. Furthermore, the IDMC identified 3 cases of mild rash and 3 cases of mild to moderate chills in temporal relationship with the administration of the product in study GENA-03. Relevance of these cases has been subject to further evaluation which did not reveal any safety concern. All other described non-inhibitory antibodies that were already present at baseline are considered to reflect a low incidence of not well understood immunological responses. As *Human-cl* rhFVIII is produced in HEK cells lacking non-human material, other immunological responses might be of theoretical relevance, only.

There were 11 additional SAEs in 8 patients in the 5 studies. None of the cases was considered to be related with the administration of *Human-cl rhFVIII*. The profile of the SAEs was considered to be in line with similar products.

One death occurred in the GENA-08 study. The patient had been diagnosed with epilepsy and had been taking an antiepileptic therapy (oxycarbazepin, 300 mg twice a day) since 1997. The CRF states acute respiratory and cardiovascular failure as the cause of death.

Routine laboratory investigations and evaluation of vital signs did not show any signals. Viral safety was described to follow accepted standards including two specific steps of viral removal. No seroconversions have been reported so far.

To date, there have been no reports of virus transmission, hypersensitivity, thromboembolism or inhibitor development related to *Human-cl rhFVIII*. However, as the database is small, these potential risks as identified in the product class were reflected in the SmPC.

A comparative AE listing reflecting AEs by SOC and PT has been provided for the clinical studies, showing accumulations of AEs typical for the paediatric age-group as upper respiratory infections or injuries. No evidence for additional age-specific signals has been identified according to this information.

All the adverse reactions reported in clinical trials in the safety database have been included in the SmPC. As adverse drug reactions (ADRs) occurred only once and the total number of studied patients was 135, the frequency was reported as “uncommon”. These were paraesthesia, headache, vertigo, dry mouth, back pain, injection site inflammation, injection site pain, non-neutralising anti-FVIII antibody positive.

Even though no inhibitors were detected, as this is recognised as an important aspect across the class, and considering the limited database, it was decided that statements on the inhibitor development are reflected in the SmPC in Sections 4.2, 4.4, 4.8 as warnings. The risk of hypersensitivity reactions is addressed as relevant warnings and contra-indications in the SmPC. Lack of knowledge in previously untreated patients (PUPs) and in children younger than 2 years is reflected in the posology section of the SmPC.

2.6.2. Conclusions on the clinical safety

Clinical safety has been analysed from the data of all clinical trial phases. Evaluation in general follows the currently valid Clinical Guideline. The presented results are considered to be acceptable.

Particular emphasis during the analysis and discussion of the safety data was given to immunogenicity and development of FVIII antibodies. Further studies and registry data (see pharmacovigilance plan and RMP) will monitor safety issues of special interest.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC advice on the submitted RMP:

PRAC Advice

The PRAC, having considered the data submitted, considered the RMP v5 can be acceptable. This advice is based on the following content of the RMP:

Safety concerns

Table 33: Summary of safety concerns

Summary of safety concerns	
Important Identified Risks	- Inhibitor development (antibodies against rhFVIII)
Important Potential Risks	- Hypersensitivity reactions, including anaphylactic reactions - Thromboembolic events - Medication error including safety in home therapy setting
Missing Information	- Safety in previously untreated patients - Children < 2 years - Immune tolerance induction (ITI) - Use in pregnant or breast feeding women

Pharmacovigilance plans

Table 34: Summary of pharmacovigilance plans

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status	Date for submission of interim or final reports/ Milestones
GENA-05 Interventional clinical study (category 3)	Investigate immunogenicity, efficacy and safety of Human-cl rhFVIII in PUPs	- Inhibitor development - Safety in PUPs, including children < 2 years - Immune tolerance induction (ITI)	Started in Q1 2013	**Milestone:** Post-approval commitment to follow up at least 100 PUPs (50 from efficacy/safety trial and 50 new) for a minimum of 100 EDs. Final report planned for 2019. Two interim analyses planned: - After 30 patients started treatment

				- After 50 patients achieved at least 50 EDs
GENA-13 Interventional clinical study (category 3)	Determine the long-term immunogenicity and tolerability of Human-cl rhFVIII	- Inhibitor development - Hypersensitivity reactions, including anaphylactic reactions	Started in Q4 2011	Final report planned for 2016.
GENA-15 Interventional clinical study (category 3)	Investigate immunogenicity, efficacy and safety of Human-cl rhFVIII in patients who completed study GENA-05 in accordance with the study protocol	- Inhibitor development	Started in Q1 2014	Final report planned for 2016.
GENA-99 Post-marketing study (category 3)	Product safety and clinical efficacy	- Inhibitor development - Hypersensitivity reactions, including anaphylactic reactions - Thrombo-embolic events - Medication error including safety in home therapy setting - Safety in children < 2 years	Planned	Final report planned for 2020. One study progress report planned two years after marketing authorisation approval.

European Haemophilia Safety Surveillance (EUHASS)	Product safety	- Inhibitor development - Hypersensitivity reactions, including anaphylactic reactions - Thrombo-embolic events - Medication error including safety in home therapy setting	Ongoing	Octapharma will receive regular product-specific reports. Relevant information included in these reports will be provided in PSURs/PBRERs.

Risk minimisation measures

Table 35: Summary of risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Inhibitor development (antibodies against rhFVIII)	Mentioned in the SmPC (Sections 4.2, 4.4 and 4.8)	None
Hypersensitivity reactions, including anaphylactic reactions	Mentioned in the SmPC (Sections 4.3, 4.4 and 4.8)	None
Thromboembolic events	Mentioned in the SmPC (Section 4.4)	None
Medication error including safety in home therapy setting	Mentioned in the SmPC (Sections 4.2 and 4.9) Mentioned in the PIL (Section 3)	None
Previously untreated patients	Mentioned in the SmPC (Section 4.2)	None
Children < 2 years	Mentioned in the SmPC (Sections 4.2, 4.4 and 4.8)	None
Immune tolerance induction (ITI)	Mentioned in the SmPC (Section 4.4)	None
Pregnant or breast feeding women	Mentioned in the SmPC (Section 4.6)	None

The CHMP endorsed this advice without changes.

2.9. User consultation

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

3. Benefit-risk balance

Benefits

Beneficial effects

Three pivotal clinical trials investigating the efficacy of simoctocog alfa for the prevention and treatment of BEs in PTPs with severe haemophilia A as well as the haemostatic efficacy for surgical procedures have been submitted. Study GENA-01 investigated the efficacy in on-demand treatment of BEs in 22 adolescent and adult patients. Study GENA-08 investigated the efficacy of simoctocog alfa for the prophylaxis of BEs and the treatment of breakthrough BEs in 32 adults. Study GENA-03 investigated the efficacy of simoctocog alfa in 59 paediatric patients between 2 and 11 years of age.

In GENA-01 in overall, 94.4% (931/986) of the BEs were treated with excellent or good efficacy (60.3% excellent, 34.1% good). A large majority of BEs required only one infusion (841/986, 91.4%) and 53 BEs (5.8%) required 2 infusions.

The mean BE rates per patient during the prophylactic treatment period at the end of GENA-08 study were 0.188/month (median 0.074; range 0–1.21) for all types of BEs. Personal efficacy assessments were available for 28 BEs. Efficacy ratings on a four-point scale were excellent or good for all BEs (71.4% (20/28) excellent, 28.6% (8/28) good).

The mean rate of all BEs in GENA-03 (prophylaxis) was 0.338 BEs/month (median 0.156; range 0–1.70) at the end of the study; the monthly rate of all BEs was lower in patients aged 2 to 5 than in those aged 6 to 12 years (0.213 vs. 0.459 BEs/month). For breakthrough BEs occurring during the study, 68.6% of BEs were treated with one infusion and 81.3% with one or 2 infusions.

Furthermore, the PK properties of simoctocog alfa were investigated in the PK part of the paediatric trial indicating that the PK profile of simoctocog alfa is comparable to that of other licensed FVIII products. Additionally, in GENA-01, the bioequivalence of simoctocog alfa and a full-length rFVIII could be demonstrated for AUC_{norm} (AUC normalised to the dose administered) in both the CHR and the OS clotting assay.

Uncertainty in the knowledge about the beneficial effects

Simoctocog alfa is the first recombinant human FVIII intended for the treatment of haemophilia A that is produced in a human cell line (i.e. HEK 293F). Furthermore no animal derived proteins are added throughout the manufacturing process or to the final product. This could potentially offer the benefit of fully human PTMs and no foreign protein material in comparison to other commercially available FVIII concentrates, which are produced in hamster cell lines (Chinese hamster ovary [CHO] and baby hamster kidney [BHK] cells); however, there are no data to support such a claim.

Efficacy in adolescents is based upon a narrow database with a gap for >14 and <18 year old patients. In study GENA-03, several patients received prophylactic doses outside the planned dose range with the majority of patients receiving doses higher than 45 IU/kg. This was reflected in the Product Information and more accurate dose recommendations for the paediatric population in the SmPC based on the study results are implemented.

No data are available for PUPs. A trial investigating the safety and efficacy of simoctocog alfa in PUPs is however currently under way (GENA-05).

Risks

Unfavourable effects

The nature and frequency of the reported AEs do not give rise to concern and do not reveal unexpected safety signals. As ADRs reported (headache, injection site pain, back pain, vertigo) occurred only once, and the database consists of 135 patients, the frequency was reported as uncommon. The size of the safety database available at the moment is in compliance with guideline requirements.

Uncertainty in the knowledge about the unfavourable effects

Due to the rareness of the disease, the safety database is relatively small, although in line with guideline requirements. Data on long-term safety will be obtained in the post-marketing phase as foreseen by the relevant guideline.

Clinical trials planned as extension studies to two of the pivotal trials (GENA-11 and GENA-13), post-marketing study GENA-99 and the data from a registry (EUHASS) are expected to bring more information on the occurrence of AEs of special interest, i.e. development of inhibitors, thromboembolic events or hypersensitivity, anaphylactic or allergic reactions and included in the pharmacovigilance plan.

Balance

Importance of favourable and unfavourable effects

Available clinical efficacy data for simoctocog alfa support that it is an efficacious new FVIII product for the prevention and treatment of BEs in PTPs with haemophilia A.

The number of included patients across all clinical trials is in accordance with guideline requirements and the safety database is considered sufficient to evaluate the tolerability of simoctocog alfa before marketing authorisation. The observed AE profile is considered similar to that of other licensed FVIII products. Importantly, no patients developed FVIII inhibitors and no thromboembolic events occurred.

Benefit-risk balance

Overall, efficacy has been established by the provided clinical data as per the requirements of relevant Guidelines. The safety profile of Nuwiq is in line with what is expected.

The benefit-risk balance in the indication of the treatment and prophylaxis of bleeding in paediatric and adult patients with haemophilia A (congenital factor VIII deficiency) is considered to be positive.

Discussion on the benefit-risk assessment

Overall, efficacy of simoctocog alfa in adult and paediatric patients with haemophilia A covering treatment of BEs, prophylaxis of BEs and prophylaxis in surgical procedures is adequately supported by submitted clinical data according to the currently valid Clinical Guideline. Furthermore, the documented safety profile is within the expected range for FVIII products. Studies detailed in the pharmacovigilance plan are expected to update the knowledge on issues of special interest in this class (development of inhibitors, hypersensitivity, anaphylactic or allergic reactions, safety in previously untreated patients).

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Nuwiq in the treatment and prophylaxis of bleeding in paediatric and adult patients with haemophilia A (congenital factor VIII deficiency) is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

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

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The MAH shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the MAH shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

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

- **Risk Management Plan**

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

An updated RMP should be submitted:

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

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

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

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that simoctocog alfa is qualified as a new active substance.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan EMEA-001024-PIP01-10-M01 and the results of these studies are reflected in the SmPC and, as appropriate, the Package Leaflet.