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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

AFSTYLA

International non-proprietary name: lonoctocog alfa

Procedure No. EMEA/H/C/004075/0000

Note

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

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List of abbreviations

ADA	Anti-drug antibodies
AE	Adverse event
ABR	Annualized bleeding rate
AsBR	Annualized spontaneous bleeding rate
aPTT	Activated partial thromboplastin time
AUC	Area under the concentration curve
BCS	Behring Coagulation System
BCS XP	Behring Coagulation System XP
BDI	Bulk drug intermediate
BU	Bethesda Units
CAT	Calibrated automated thrombogram
CFU	Colony forming units
cGMP	(Current) good manufacturing practice
CHO	Chinese hamster ovary
ChS	Chromogenic substrate activity assay
CI	Confidence interval
CL	Clearance
Cmax	Observed maximum plasma concentration
CPP	Critical process parameter
CPV	Continued Process Verification
CQA	Critical quality attribute
CSL627	Sponsor-assigned drug code for rVIII-SingleChain
CSR	Clinical Study Report
CT	Clotting time
CV	Coefficient of variation
DHFR	Dehydrofolate reductase
DNA	Deoxyribonucleic acid
DP	Drug product
DS	Drug substance
EDs	Exposure Days

EMA	European Medicines Agency
ELISA	Enzyme-linked immunosorbent assay
EoP	End-of-production cell bank
EP	European Pharmacopoeia
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FMEA	Failure modes and effects analysis
FVIII	Human coagulation factor VIII
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
HCP	Host cell protein
HPLC	High performance liquid chromatography
IAC	Immuno-affinity-chromatography (FactorVIIISelect)
IPAC	In-process acceptance criterion
IPC	In-process control
i.v.	Intravenous
ICH	International Conference on Harmonization
Ig	Immunoglobulin
IgA	Immunoglobulin isotype A
IgG	Immunoglobulin isotype G
IgM	Immunoglobulin isotype M
IR	Incremental recovery
IU	International Units
IV	Intravenous
ko	Knockout
MCB	Master cell bank
MedDRA	Medical Dictionary for Regulatory Activities
Mio IU	Million International Units
n.a.	Not applicable
NOAEL	No Observed Adverse Effect Level
NONMEM	Non-linear mixed effects model

NOR	Normal operating range
NHP	Normal human plasma
OD	Optical density
OS	One-stage clotting assay
PCS	Process control strategy
pd	plasma-derived
PPCB	Post-production cell bank
PPQ	Process performance qualification
PR	Interval between P wave and onset QRS
PRI	Product-related impurity
PRS	Product-related substance
PS80	Polysorbate 80
PT	Prothrombin time
PVMP	Process validation master plan
QbD	Quality by Design
RCB	Research cell bank
RP-HPLC	Reverse phase HPLC
RPN	Risk priority number
rFVIII	Recombinant Coagulation Factor VIII
rVIII-SingleChain	CSL627
SAE	Serious adverse event
SD	Standard deviation
S/D	Solvent/detergent (TnBP/PS80)
SEC	Size exclusion chromatography
SMQ	Standardised MedDRA Query
SOC	System Organ Class
SOP	Standard operating procedure
ST	ST interval
t1/2	Half-life
$t_{1/2\beta}$	Terminal half-life
TEAE	Treatment-emergent adverse event
TEE	Thromboembolic events

TESAE	Treatment-emergent serious adverse event
Tmax	Time to observed maximum plasma concentration
TnBP	Tri(n-butyl)phosphate
USP	United States Pharmacopoeia
VWF	von Willebrand factor
WCB	Working Cell Bank
WFH	World Federation of Haemophilia
WHO	World Health Organization

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CSL Behring GmbH submitted on 4 December 2015 an application for marketing authorisation to the European Medicines Agency (EMA) for AFSTYLA, 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 in patients with haemophilia A (congenital factor VIII deficiency).

AFSTYLA 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 lonoctocog 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/or 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/0227/2015 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0227/2015 not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

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

Applicant's request(s) for consideration

New active Substance status

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

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Jan Mueller-Berghaus Co-Rapporteur: Tuomo Lapveteläinen

- The application was received by the EMA on 4 December 2015.
- The procedure started on 31 December 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 21 March 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 21 March 2016. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 31 March 2016.
- During the meeting on 28 April 2016, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 15 July 2016.
- The following GCP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:
  - A GCP inspection at two clinical sites (Thailand and Malaysia) and the sponsor site (Germany) between 29 March 2016 and 20 May 2016. The outcome of the inspection carried out was issued on 04 July 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 31 August 2016.
- During the PRAC meeting on 2 September 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. .
- During the CHMP meeting on 15 September 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 11 October 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 26 October 2016.
- During the meeting on 7-10 November 2016, 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 Afstyla on 10 November 2016.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Haemophilia A is a rare and serious, X-linked, recessive bleeding disorder that predominantly affects males and is characterized by a deficiency of FVIII. In patients with haemophilia A, the primary platelet-driven hemostasis is not affected, but generation of a stable, fibrin-rich clot is defective because

inadequate amounts of thrombin are generated. Affected patients suffer from both spontaneous, non-traumatic bleeding episodes as well as substantially prolonged bleeding episodes upon injury. Rarely, life-threatening bleeding may also occur. Patients exhibit variable clinical phenotypes depending on the extent of residual activity (%) of the deficient FVIII that is used to classify the disease severity (WFH, 2012):

- >1% FVIII activity: severe haemophilia A
- 1% to 5% FVIII activity: moderate haemophilia A
- 5% to 40% FVIII activity: mild haemophilia A

Patients with severe haemophilia A bleed spontaneously into joints and muscles, which often results in permanent, disabling joint damage.

Afstyla is indicated for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA can be used for all age groups.

2.1.2. Epidemiology

The overall reported number of haemophilia A patients estimated in the 2013 survey by the World Federation of Haemophilia (WFH) included 107 countries with a total population of 6,461,067,861 and identified 140,313 people with haemophilia A (2.2 per 100,000 individuals). There are currently approximately 30,000 patients in the EU with a mean prevalence of approximately 0.6 patients per 10,000.

Haemophilia A is inherited as an X-linked recessive trait and the main risk factors are therefore family history and a carrier mother. Approximately 30% of patients have no family history of the disease; their disease is presumably caused by new mutations.

2.1.3. Biologic features, aetiology and pathogenesis

The factor VIII/von Willebrand factor complex consists of two molecules (factor VIII and von Willebrand factor) with different physiological functions. When infused into a haemophiliac patient, factor VIII binds to von Willebrand factor in the patient's circulation. Activated factor VIII acts as a cofactor for activated factor IX, accelerating the conversion of factor X to activated factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot can be formed.

2.1.4. Clinical presentation, diagnosis

Haemophilia A manifests as profuse bleeding into the joints and muscles or internal organs, either spontaneously or as the result of accidental or surgical trauma. Recurrent joint bleeding can lead to chronic arthropathy, pain, and loss of function (Bolton-Maggs and Pasi, 2003). The majority of bleeding occurs internally into joints, most commonly hinged joints such as the ankles, knees, and elbows. Serious bleeds also occur in muscles, especially in deep compartments such as the iliopsoas, calf and forearm, and in the mucous membranes in the mouth, gums, nose, and genitourinary tract. Less frequently, life threatening bleeds can occur in or around vital areas or organs such as the gastrointestinal system or enclosed areas like the intracranial or intracerebral spaces. The approximate frequencies of bleeds at the different sites are: 70 to 80% in joints (hemarthrosis), 10 to 20% in muscle, 5 to 10% in the central nervous system, and < 5% for bleeds at all other sites (Srivastava et al., 2013).

2.1.5. Management

Standard treatment for haemophilia A patients is the replacement of the missing protein by infusion of exogenous FVIII concentrates (as plasma-derived FVIII [pdFVIII] or recombinant FVIII [rFVIII] concentrates). Treatment regimens are either on-demand therapy (given when a bleed occurs) or prophylaxis (which consists of regular infusion of FVIII given every 2 to 3 days to prevent bleeding). In the short term, prophylaxis can prevent spontaneous bleeding and in the long term, prophylaxis can prevent bleeding into joints that will eventually lead to debilitating arthropathy.

Prior to the introduction of clotting factor concentrates in the 1960s, the prognosis for haemophilia A patients was poor, average life expectancy being 15 to 25 years. Major advances in the safety of clotting factor products, including the availability of rFVIII concentrates, the availability of comprehensive haemophilia A treatment centres, the institution of routine prophylaxis, the introduction of home treatment, as well as the active roles that patients take in self-advocacy, have enabled patients with haemophilia A to lead a “close to normal” life.

About the product

Afstyla is formulated as a sterile, non-pyrogenic, preservative-free, lyophilized, white to slightly yellow powder or friable mass intended for intravenous administration provided in a single-use vial.

It is a recombinant human protein that replaces the missing coagulation factor VIII needed for effective hemostasis. Afstyla is a single polypeptide chain with a truncated B-domain that allows for a covalent bond to link the factor VIII heavy and light chains. Afstyla has demonstrated a higher VWF affinity relative to full-length rFVIII. VWF stabilizes factor VIII and protects it from degradation. Activated Afstyla has an amino acid sequence identical to endogenous FVIIIa.

Each single-use vial contains nominally 250 IU, 500 IU, 1000 IU, 1500 IU, 2000 IU, 2500 IU or 3000 IU (International Units) of rVIII-SingleChain for reconstitution with liquid diluent (Sterile Water for Injection), which is provided in glass vials (2.5 ml sWFI: 250 IU, 500 IU, 1000 IU; 5.0 ml sWFI: 1500 IU, 2000 IU, 2500 IU, 3000 IU).

Type of application and aspects on development

This application has been submitted as an Article 8.3 of Directive 2001/83/EC as a new active substance, lonoctocog alfa. Afstyla contains the rFVIII protein

Afstyla is indicated for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA can be used for all age groups.

The posology for On Demand Treatment is proposed as follows:

The calculation of the required dose of factor VIII is based on the empirical finding that 1 International Unit (IU) factor VIII per kg body weight raises the plasma factor VIII activity by 2 IU/dl.

The required dose is determined using the following formula: Dose (IU) = body weight (kg) x Desired factor VIII rise (IU/dl or % of normal) x 0.5 (IU/kg per IU/dl).

The amount to be administered and the frequency of administration should always be oriented to the clinical effectiveness in the individual case.

In the case of the following haemorrhagic events, the factor VIII activity should not fall below the given plasma activity level (in % of normal or IU/dl) within the corresponding period. The following table can be used to guide dosing in bleeding episodes and surgery:

Degree of haemorrhage / Type of surgical procedure	Factor VIII level required (%) (IU/dl)	Frequency of doses (hours) / Duration of therapy (days)
Haemorrhage		
Early haemarthrosis, muscle bleeding or oral bleeding	20 - 40	Repeat injection every 12 to 24 hours. At least 1 day, until the bleeding episode as indicated by pain is resolved or healing is achieved.
More extensive haemarthrosis, muscle bleeding or haematoma	30 - 60	Repeat injection every 12 to 24 hours for 3-4 days or more until pain and acute disability are resolved.
Life threatening haemorrhages	60 - 100	Repeat injection every 8 to 24 hours until threat is resolved.
Surgery		
Minor surgery including tooth extraction	30 - 60	Inject every 24 hours, at least 1 day, until healing is achieved.
Major surgery	80 - 100 (pre- and postoperative)	Repeat injection every 8 to 24 hours until adequate wound healing, then therapy for at least another 7 days to maintain a factor VIII activity of 30% to 60% (IU/dl).

For long term prophylaxis, the recommended starting regimen is 20 to 50 IU/kg of AFSTYLA administered 2 to 3 times weekly. The regimen may be adjusted based on patient response.

Previously untreated patients:

The safety and efficacy of AFSTYLA in previously untreated patients have not been established. No data are available.

Paediatric patients:

The recommended starting regimen in children (0 to <12 years of age) is 30 to 50 IU per kg of AFSTYLA administered 2 to 3 times weekly. More frequent or higher doses may be required in children <12 years of age to account for the higher clearance in this age group. For adolescents of 12 years of age and above, the dose recommendations are the same as for adults.

The development of Afstyla is in agreement with the Paediatric Investigation Plan (PIP); PIP P/0227/2015, it is noted that some measures were deferred.

2.2. Quality aspects

2.2.1. Introduction

Afstyla finished product is a sterile, preservative-free, lyophilized powder and solvent (water for injections (WFI)) for solution for i.v. administration supplied in single-use vials. Each single-use type I glass vial

contains nominally 250 IU, 500 IU, 1000 IU, 1500 IU,2000 IU, 2500 IU or 3000 IU (International Units) of recombinant, single chain coagulation factor VIII (rVIII-SingleChain) for reconstitution with liquid diluent (Sterile Water for Injection), which is provided in type I glass vials (2.5 ml sWFI: 250 IU, 500 IU, 1000 IU; 5.0 ml sWFI: 1500 IU, 2000 IU, 2500 IU, 3000 IU).

2.2.2. Active Substance

General information

The active substance (AS) in Afstyla is "recombinant, single chain coagulation factor VIII", which is expressed and secreted by CHO cells.

The active substance is a single-chain recombinant Factor VIII (rVIII-SingleChain) construct where most of the B-domain and 4 amino acids of the adjacent acidic a3 domain were removed(amino acids 765 to 1652 of full-length FVIII, including the furin-cleavage site). The newly formed linkage of the heavy and light chain of FVIII introduces a new N-glycosylation site. It has 1444 amino acids in a single chain glycopeptide with a molecular weight of approximately 170 kDa.

After activation by thrombin and removal of the (residual) B- and a3-domain, the activated rFVIII (rFVIIIa) molecule formed has an amino acid sequence identical to FVIIIa formed from endogenous, full length, FVIII.

The three dimensional structure of rVIII-SingleChain is stabilized by eight disulphide bridges. The presence of three "free thiols" has been confirmed. Regarding glycosylation, rVIII-SingleChain contains six N-glycosylation sites, but was found to have limited O-glycosylation. rVIII-SingleChain also exhibits nearly complete sulphation of its tyrosine residues.

Manufacture, characterisation and process controls

The manufacturing process of the active substance consists of an upstream cell culture process and a downstream purification process. The manufacturing process has been described in sufficient detail in line with ICH Q11. For all sites involved in manufacture and testing of rFVIII-SingleChain bulk drug intermediate (BDI) and active substance compliance to cGMP has been confirmed by respective certificates issued by EU Competent Authorities.

The active substance manufacturing process consists of 12 steps, including cell culture, column chromatography purification and concentration unit operations. Additionally there are 3 validated virus removal steps two of which are dedicated.

The first steps cover the WCB vial thaw, cell expansion and bioreactor cultivation. Obtained harvests are concentrated and stored. The hold time of the harvest bags has been adequately justified. Two chromatography steps for primary capture, concentration and buffer exchange, and a solvent/detergent (S/D) virus inactivation step are performed to obtain the BDI.

The downstream purification process consists of 3 chromatography steps for further purification and a virus nanofiltration step.

The upstream part of the manufacturing process has been described in sufficient detail. It can be concluded that the rFVIII-SingleChain is produced in a state-of-the-art, well-controlled and efficient cell culture process.

The downstream purification process follows a common purification strategy, consisting of chromatography and filtration steps and two virus-removal steps. Full-scale validation studies on the re-use and regeneration of chromatography columns have been provided.

Control of Materials

In line with ICH Q5B, the source and history of the CHO cell line into which the rFVIII gene transcript was cloned and the characteristics of the mammalian expression vector have been described. The parental cell line is commonly used for the expression of foreign proteins. The rationale for particular characteristics of the rFVIII construct has been described: a stable and efficiently expressed construct was selected by analysing structure-function relationships of various constructs. Most suitable characteristics were a B-domain-deleted construct with reduced proteolytic degradation, intact thrombin cleavage sites and linker sequence for full activation and coagulation activity, good Von Willebrand factor (vWF) binding, and low immunogenic risk of the linker region. The rationale for the introduction of an additional N-glycosylation site in the linker region in order to reduce the antigenicity risk has been provided.

The CHO host cell line used to create the rVIII-SingleChain cell substrate was confirmed to be free from contamination by viruses, bacteria (including mycoplasma) and fungi (moulds and yeasts). Cell bank characterisation test reports have been provided and the integrity of the final expression vectors was verified.

In line with ICH Q5A (R1) "Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin" and Q5D the comparability of the MCB, the WCB and the post production cell bank (PPCB) with respect to identity, the absence of adventitious agents and the genetic stability has been demonstrated.

A two-tiered cell banking system has been established using a MCB and a WCB. The WCB was found to be free of adventitious agents, genetically stable, and comparable to the underlying MCB.

The genetic stability and productivity beyond the *in vitro* cell age has been demonstrated on an end-of-production (EoP) cell bank and a post-production cell bank (PPCB).

Most raw materials used in the manufacture of BDI and of active substance are of Ph. Eur. quality. For non-compendial raw materials satisfactory specifications have been presented. Beside the host cell line of hamster origin no other raw material of human or animal origin is used in the purification and formulation processes of rFVIII-SingleChain.

All chromatography steps are performed in the absence of any human-or animal-derived raw material and each chromatography resin in use is dedicated to rFVIII-SingleChain. All materials used in the manufacture of filters are either animal free or in compliance with *Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (*EMA/410/01, current version).

Control of Critical Steps and Intermediates

The rVIII-SingleChain active substance process control strategy was developed using a systematic, scientific, and risk-based approach. The control strategy was developed to appropriately control sources of process variability such that the desired process performance and product quality (i.e., Critical Quality Attributes (CQA)) are consistently achieved. This included process parameters classification and process parameter risk assessment. Respective risk assessment reports have been provided. In their process control strategy (PCS) "critical steps" have been defined as those that contain critical process parameters (CPP), in-process controls (IPC) and /or in-process acceptance criteria (IPAC). CPP, IPC and IPAC have

been defined in accordance with ICH Q8(R2) and ICH Q6B. The limits/acceptance criteria are based on statistical analysis of historical data, process characterisation and process capability.

The monitoring parameters, the test methods and their acceptance criteria appear suitable to control the BDI.

Process validation

The process performance qualification was designed to verify the process control strategy by demonstrating that the process, when operated within the defined ranges, produces rVIII-SingleChain AS that consistently meets all in-process controls, in process acceptance criteria, sampling/testing requirements, and release specifications.

Process validation master plans (PVMPs) have been prepared which adopted very similar process validation methodology and included implementation of the lifecycle approach to process validation. The master plans describe the activities that are included in each stage of the rVIII-SingleChain AS process validation program including Stage 1 (Process Design), Stage 2 (Process Qualification) and Stage 3 (Continued Process Verification). The process validation uses small scale and full scale studies and follows current FDA and EMA guidance on process validation.

The outcomes of the validation studies demonstrate that the manufacturing process operates at all steps consistently within the proposed acceptance ranges and provides product that meets pre-set acceptance criteria.

In ancillary validation studies the mixing steps, intermediate hold times, chromatography resin and membrane life times, as well as material shipment have been successfully validated. It has been sufficiently demonstrated that the small scale experiments on the chromatography steps are representative for the commercial scale process, including sanitization procedures.

Manufacturing Process Development

Two manufacturing scales can be distinguished: material from pilot scale cell culture was used for preclinical and early clinical studies, commercial scale bioreactor material for later stage clinical development. Comparability of the material from the different development stages (plot and commercial scale) has been demonstrated. No significant differences have been observed in the cell culture process performance. Comparison of in-process quality attributes during the downstream purification process at pilot and commercial scale indicated that despite some differences at individual steps the results are comparable.

Characterisation

A detailed characterisation of rFVIII-SingleChain has been presented determining primary, secondary and higher order structures, and studying functional properties in comparison to commercial rFVIII and plasma-derived FVIII (pdFVIII). The expected amino acid sequence of the fusion construct has been fully confirmed.

From functional characterizations it is apparent that rFVIII-SingleChain has a much lower FVIII activity in the OS clotting assay compared to the chromogenic substrate assay. The ratio OS/ChS assay activity is ~0.5 and substantially lower than in other rFVIII products or in pd FVIII. The differences in FVIII activity between rFVIII-SingleChain and competitor rFVIII products is more pronounced when using the OS assay, compared to the ChS assay.

A respective statement is included in the SmPC pointing out that the chromogenic substrate assay should be used during clinical monitoring, if available. If the OS assay is used, the results are approximately 45

% lower than the ChS results. It is indicated in the SmPC that results from OS and ChS assay can be aligned by multiplying the OS results by the factor 2. For practical reasons in daily clinical use, the Applicant decided to round the initially suggested factor 1.8 to 2, and amended the SmPC accordingly.

The levels of product-related impurities present in commercial scale AS batches were monitored by several methods that are part of the AS testing and by additional tests for a limited number of batches. In general, a very low impurity level was observed. The formation of product related impurities is convincingly controlled.

Process related impurities have been investigated in detail using state-of-the-art methodology. Major and minor process-related impurities have been identified and characterised by sensitive analytical methods. It is demonstrated that the manufacturing process effectively and consistently reduces impurities, and that rVIII-SingleChain AS contains only low level of process-related impurities which seem to be of no risk for the quality, safety and efficacy of the product.

Specification

The control of the active substance is performed in line with ICH Q6B. An adequate set of active substance release specification (Table 1) has been established to cover structural and functional characteristics, critical impurities and the safety of rFVIII-SingleChain. The specifications have been established based on statistical evaluation of historical batch analysis data from AS batches of pilot and commercial scale. Moreover, process and assay capability as well as patient safety and clinical experience were taken into consideration when establishing release specifications. Batch analysis data from pilot and commercial scales AS batches indicate that the AS release specification is consistently met.

Considerable variation in FVIII one stage assay results is seen in active substance batches. This has been adequately discussed from a quality and safety point of view.

For all analytical test procedures descriptions have been provided. The methods have been validated in line with ICH Q1(R2).

Reference Standards

For the FVIII potency an adequate characterisation of the working standard has been performed. A list of acceptance criteria for current and future reference materials has been specified in case a replacement of material is needed. The formulation (dilution) and the storage conditions of the reference standard have been described, including the container closure system. A re-test period has been specified for the potency standard or any other standard, including acceptance criteria for the declared potency value. The reference standard has been calibrated against the WHO International Standard (IS).

The initial potency assignment for new reference standards in relation to the current IS or a new IS and the acceptance criteria for the establishment of new reference standards have been described in sufficient detail. The strategy for replacement of the working standard and the proposed stability monitoring program can be accepted. Regarding the establishment of a new WRS or a new IS, the proposed strategy ensures that the consistency in the labelling of the clinical batches and the commercial batches during the product life cycle is guaranteed.

Stability

The stability of the active substance under long-term has been investigated in line with ICH Q5C “Stability testing of Biotechnological/Biological products”. Real time/real temperature data are presented for commercial scale AS batches. Stability data provided no evidence that quality parameters show a negative trending and the proposed shelf life can be accepted.

The active substance is either processed immediately after the virus filtration step or is stored for the claimed shelf life at the proposed storage conditions. The storage containers for the bulk active substance are in compliance with compendial requirements (Ph. Eur. and USP).

Adequate details on the storage containers used for the hold time stability studies of the bioreactor harvests and chromatography eluates have been provided including data that demonstrate an acceptable extractable and leachable level.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is supplied as a single-use type I glass vials of 6 mL (250 IU, 500 IU, 1000 IU of lonoctocog alfa per vial) and 10 mL (1500 IU, 2000 IU, 2500 IU, 3000 IU of lonoctocog alfa per vial) intended for intravenous (i.v.) injection. The vials are closed with a bromobutyl rubber stopper and sealed with an aluminum overseal closure using different colors for the various strengths.

The active substance rVIII-SingleChain is formulated in a histidine buffer containing stabilizers and a bulking agent. The formulation contains the excipients L-histidine, polysorbate 80, calcium chloride dihydrate, sodium chloride and sucrose. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.

The lyophilized finished product is reconstituted with sterile water for injections. For the 250 IU, 500 IU and 1000 IU strengths 2.5 ml of sWFI and for the 1500 IU, 2000 IU, 2500 IU and 3000 IU strengths 5.0 ml of sWFI are used.

The development of the finished product manufacturing process occurred in two key stages. The pilot scale process supplied finished product for preclinical pharmacology and toxicology studies as well as early clinical studies and stability studies (clinical study CSL627_1001). The commercial scale process (250 IU, 500 IU, 1000 IU, 2000 IU and 3000 IU) supplied finished product for further clinical studies (CSL627_1001, CSL627_3001, CSL627_3002), stability studies and PPQ studies.

The first stage of the finished product development at the pilot scale comprised the development of the 1000 IU strength. Later on during scale-up to the commercial scale production process, the additional strengths including 250 IU, 500 IU, 2000 IU and 3000 IU were implemented.

The 1500 IU and 2500 IU strengths were introduced following completion of the development program for the initial five strengths (250 IU, 500 IU, 1000 IU, 2000 IU and 3000 IU). The formulation development studies resulted in a formulation procedure allowing all strengths to be formulated using the same formulation buffer. The different strengths are formulated by addition of varying amounts of the formulation buffer to obtain the desired strength. The formulation buffer developed is free of animal-derived materials.

The unit operations were consistent throughout development. Process changes included mostly scale-up changes and also process optimizations were performed. The product fill volumes (2.5 ml: 250 IU, 500 IU, 1000 IU; 5,0 ml: 1500 IU, 2000 IU, 2500 IU, 3000 IU) as well as the respective lyophilisation cycles were adapted depending on the strength.

Two fill volume-specific lyophilitzation cycles were developed for the 250 IU, 500 IU and 1000 IU strengths as well as for the 1500 IU, 2000 IU, 2500 IU and 3000 IU strengths (5,0 ml). The development studies of the lyophilisation process lead to a robust commercial freeze-drying process which yields a product with consistent quality attributes.

The finished product formulation was shown to be stable and no overages are applied during manufacture of the finished product. The stability data support compatibility of the active substance with the finished product excipients. The lyophilized finished product is a white whole to slightly yellow powder.

No major process changes regarding the finished product manufacturing process occurred during the life cycle from development to commercial scale manufacturing, indicating that the Phase I, Phase I/II and Phase III clinical trial material as well as the commercial product are manufactured by almost the same process and are of comparable quality. No formulation changes were performed.

Manufacture of the product and process controls

The manufacturing of the finished product consists of formulation and sterile filtration, filling, lyophilization, capping and crimping, labelling and packaging. A detailed process flow diagram including a summary of the in-process controls (IPC) and in-process acceptance criteria (IPAC) tests conducted at each stage of the manufacturing process and interim storage conditions has been provided.

The active substance from Step 12 is the starting material for the finished product manufacturing process. The active substance can be stored frozen at ≤-65 °C or used immediately.

Reprocessing

The validation reports supporting reprocessing for one step have been provided.

In addition, the process control strategy as well the controlled parameters (input and output parameters) for the different operation units were described in detail. The Risk Priority Number (RPN) for each input parameter was calculated. These scores were used to identify potential high risk parameters that needed specific focus in validation studies. The process is considered appropriately controlled.

Control of Critical Steps and Process Control Strategy

During process development, a risk-based and science-based approach was used to reveal the relationship between manufacturing process variables and product quality attributes.

The Process Control Strategy ensures consistent manufacture of finished product that meets its defined critical quality attributes (CQAs). In developing this strategy, all process parameters were evaluated with regard to risk of failure, taking into account scientific rationale, data from process characterization studies and historical manufacturing data. The suitability of the control strategy was confirmed during PPQ.

A Process Validation Master Plan was developed which defines the strategy to ensure a reliable and consistent manufacturing process capable of delivering product of appropriate quality.

The finished product manufacturing process was qualified according to a prospective PPQ protocol which defined the sampling, analytical testing and acceptance criteria for each process step. The PPQ results have been presented. Taken together, all study objectives of the PPQ were met and the defined PCS was successfully validated for the manufacture of finished product.

Validation data were presented covering the finished product manufacturing steps including formulation, sterile filtration, filling and lyophilization. In addition, information on the hold times as well the reprocessing at the sterile filtration step has been provided.

In general, the process validation strategy provided is acceptable and follows a Continuous Process Verification approach as described in the relevant guidelines on process validation for finished products (EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev1 – Process Validation; ICH Q8 (R2 – Pharmaceutical Development)).

Moreover, a Continued Process Verification (CPV) plan is in place to ensure the validated state of the finished product manufacturing process throughout the commercial life cycle.

Product specification

The finished product specification used for release/shelf life testing is considered appropriate including adequate tests for integrity, potency, purity and quality. The acceptance criteria for the control parameters are based on historical batch data (pilot scale and/or commercial scale). The specification parameters and their acceptance criteria are adequate and meet the requirements of guideline ICH Q6B.

Analytical methods

Adequate detail on the analytical procedures and their validation has been provided. Batch analysis

Based on the batch analysis data presented for the commercial batches (validated process), acceptance criteria for the parameter protein concentration were introduced. In addition, the limits (release and shelf life) for the parameter HMWC were revised.

The information provided on the batch analysis data as well as on the characterization of the process- and product-related impurities is considered appropriate. Product-derived impurities, e.g. aggregates and degradation products are controlled in both the active substance and the finished product manufacturing process. Specifications regarding product-related impurities were established. No new process-related impurities were identified in the finished product when compared to the active substance.

Potency Reference Standard

The 8th International Standard for FVIII concentrate (NIBSC 07/350) is used as Primary Reference Standard. A product-specific Working Reference Standard was established which was calibrated against the 8th International Standard for FVIII concentrate (NIBSC 07/350).

A consistent potency declaration from early clinical trials throughout the commercial life cycle is in place.

The general strategy for the establishment, the calibration and the stability monitoring of the Reference Standards (PRS and WRS) is discussed in more detail in the active substance section.

Stability of the product

The stability of the finished product was investigated for all strengths. Long-term stability studies for commercial scale batches at +5°C, +25°C, 30°C and +40°C (stress condition) were performed in order to monitor the physical, chemical and biological integrity of the finished product over time. Temperature shift studies were also included.

The batches included into the stability studies used the final container closure system. The container closure system consists of 6 ml and 10 ml type I glass vials and a bromobutyl rubber stopper. The stoppers are secured by combination caps consisting of an aluminum crimp cap with a concentric hole and an integrated polypropylene plastic disc. The packaging materials for storage of the finished product are in compliance with Ph. Eur. and USP requirements and are suitable for the intended use. The silicone oil compatibility of closures with the product has been discussed.

Studies regarding leachables and extractables were presented. The evaluations assessed non-volatile (LC-MS) and semi-volatile substances (GC-MS) as well as inorganic elements (ICP-MS). In addition, the issue of possible leachables and extractables derived from the bromobutyl rubber stopper was addressed, and the risk assessment was provided.

Based on the currently available stability data presented the finished product was shown to be stable at the various storage conditions. In addition, the available stability data for the reconstituted product show physicochemical stability for up to 48 hours at maximum +25 °C. From a microbiological point of view the reconstituted product should be used immediately after reconstitution.

A photostability study was performed according to ICH 1B. The data presented indicate that the finished product must be stored protected from light. Accelerated stability studies at +40 °C and forced degradation studies showed changes in finished product as expected and confirmed that the assays used in the stability program are stability indicating.

The stability studies provided are in general acceptable. The proposed shelf life claim is supported by suitable stability data for the 250 IU, 500 IU and 1000 IU strengths.

The currently available real time/real temperature data for commercial scale batches support the proposed shelf life of 3 years for all dosage strengths at 2 °C to 8 °C including a single period of up to 3 months storage at +25 °C as well as the reconstitution stability as claimed in the SmPC.

Comparability exercise for finished medicinal product

The comparability of finished product manufactured using the pilot or commercial scale process was evaluated. Analytical comparability studies were performed in order to show that the early non-clinical and clinical data generated using material from the pilot scale process was supportive of the commercial scale process material which was used for further clinical studies.

The comparability of the performance of the manufacturing processes of the finished product at the pilot scale and the commercial scale was assessed by comparing product quality attributes and process performance attributes throughout the manufacturing process. Comparability was also assessed by comparison of the analytical specification and characterization results obtained for the Bulk Drug Intermediate (BDI), the active substance and the finished product.

Finished product comparability was assessed by product safety attributes, filling and lyophilization performance attributes and product quality attributes.

The comparison demonstrated that material manufactured using the pilot scale process was comparable to material manufactured using the commercial scale process.

No major process changes regarding the finished product manufacturing process occurred during the life cycle from development to commercial manufacturing, indicating that the Phase I, Phase I/II and the Phase III clinical trial material of the commercial scale process as well as the commercial product are manufactured by the same process and are of comparable quality.

In conclusion, the comparability studies are in compliance with the guideline ICH Q5E.

Adventitious agents

TSE compliance

Fetal bovine serum (FBS) was only used before generation MCB. Compliance with the TSE Guideline (EMEA/410/01, current version) has been sufficiently demonstrated.

Virus safety

The recombinant therapeutic protein is produced in a cell culture medium free of animal or human-derived components. MCB and WCB and cells at the limit of *in vitro* cell age have been sufficiently

screened for adventitious and endogenous viruses. The tests failed to demonstrate the presence of viral contaminants.

Only A-type retrovirus like particles have been identified. CHO cells are well known to produce endogenous retrovirus-like particles. The presence of retroviral particles in CHO-cells is not of concern since there is excess reduction capacity for retroviral particles within the manufacturing process.

The purification process includes three steps (chromatography, S/D treatment, and serial nanofiltration) which have been validated for their virus removal capacity of enveloped and non-enveloped viruses. Sufficient overall virus safety has been demonstrated.

Finished product - Solvent WfI in a vial

The information provided for the solvent shows that the sWFI is manufactured under GMP compliant conditions using a validated process.

sWFI is provided as 2.5 ml and 5.0 fill sizes in 6 ml type I glass vials which are closed with a chlorobutyl stopper. sWFI produced by CSL Behring in Marburg, Germany meets Ph. Eur. requirements for sterile WFI and has a shelf life of 60 month when stored at +25 (± 2)°C / 60 (± 5) % RH. The proposed shelf life is supported by real time stability data.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Active substance

Information on development, manufacture and control of the active substance has been presented in a satisfactory way. The results of tests carried out indicate that the active substance is manufactured in a validated and well-controlled process, including two virus-reduction steps, yielding an active substance of consistent quality and safety. No material of animal or human origin is used in the purification and formulation of the active substance, except the CHO cell line. A relatively high discrepancy is seen between results generated with the OS clotting assay and the chromogenic substrate assay (ChS/OS ratio ~1.8), indicating that the compendial ChS assay should be used for monitoring rFVIII-SingleChain. The use of a correction factor of 2 when using a one stage clotting assay during clinical monitoring in order to align results with chromogenic assay results appears justified from a quality point of view. No influence of vWF on the outcome of FVIII potency of rFVIII-SingleChain was found, except at very low vWF level.

Sufficient details on the different reference materials used in the release testing of active substance and finished product have been presented. The strategy to use the WHO IS as PRS and not to implement a product-specific PRS, calibrated against the WHO IS, can be accepted. In addition, adequate details have been presented: on the initial calibration of the WRS against the WHO IS; on the strategy for re-calibration of a WRS once a new WHO IS becomes available or when replacing a WRS and on the WRS stability monitoring program. It has been also adequately discussed how the consistency in the labelling of the clinical batches and the commercial batches during the product life cycle is guaranteed.

Finished product

The finished product is manufactured by a process that operates reproducibly within established parameters leading to a product that meets its pre-determined quality attributes. The process was validated in a continuous process verification approach and the respective Master Validation Plan was provided.

An adequate set of control parameters, IPCs, and IPACs including the limits and acceptance criteria was established based on a risk analysis to control the quality of the finished product. The chosen control strategy is considered appropriate.

Several specification parameters including the total protein amount and the HMWC (SE-HPLC) were revised based on the batch analysis data available for the commercial and validated process.

The currently available real time/real temperature data is acceptable in order to support the claimed shelf life and storage temperature as well as the reconstitution stability as claimed in the SmPC.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Active substance

The information on the development, manufacture, process validation and control of the active substance has been described in sufficient detail. It can be concluded that the active substance manufacturing process operates under well-controlled conditions leading to an active substance of consistent quality and safety. The active substance has been intensively characterized, both on pilot and commercial scale batches. Efficient removal of product-and process-related impurities is demonstrated. The active substance release specifications are adequately set; a release specification for individual N-glycans (neutral, mono-, di-, tri-, and tetrasialylated glycans) will be established post-approval, once sufficient data are available. As well the active specification for monomer peak in RP-HPLC of ≥75% will be re-evaluated and adjusted, if needed (Recommendations).

Finished product

Overall, the data presented indicate that the finished product is manufactured by a validated, controlled process taking into consideration relevant guidance documents. Batch release data confirm a product of consistent quality. The finished product manufacturing process operates under validated conditions to yield a finished product of consistent quality.

In conclusion, based on the review of the quality data provided, the marketing authorisation application for Afstyla is approvable from the quality point of view.

2.2.6. Recommendation(s) 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 some points for further investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

2.3.2. Pharmacology

The pharmacodynamic studies were performed *in vitro* and *in vivo*. While the *in vitro* studies were performed in the species used for toxicology studies, i.e. rat and monkey, with the aim to show pharmacodynamic efficacy in these species, the *in vivo* studies were performed in FVIII deficient mice (FVIII knock-out [ko] mice, haemophilia A mice), which have demonstrated similar deficiencies in their coagulation system compared to human patients and show an increased bleeding risk which is also seen in haemophilia A patients.

The safety pharmacology investigations were included in the toxicology studies as appropriate, and complemented by specific studies as necessary.

Table 1: Overview of pharmacological studies

Type of Study	Species and Strain	Method of Administration
Primary pharmacodynamics	Rat/	In vitro
Primary pharmacodynamics	Cynomolgus Monkey/ Macaca fascicularis	In vitro
Primary pharmacodynamics	Mouse/ FVIII ko	i.v.
Primary pharmacodynamics	Mouse/ FVIII ko	i.v.
Primary pharmacodynamics	Mouse/ FVIII ko	i.v.
Primary pharmacodynamics	Mouse/ FVIII ko	i.v.
Subchronic toxicity in rats / Neurobehavioral	Rat/	i.v.
Subchronic toxicity in monkeys / Cardiovascular	Cynomolgus Monkey/ Macaca fascicularis	i.v.
Safety Pharmacology in anaesthetized dogs / Cardiovascular and Respiratory	Dog/ Beagle	i.v.
Safety Pharmacology in a telemetered dog / Cardiovascular	Dog/ Beagle	i.v.
Safety Pharmacology in telemetered monkeys / Cardiovascular	Cynomolgus Monkey/ Macaca fascicularis	i.v.

Primary pharmacodynamic studies

***In vitro* studies**

Study: Pharmacodynamic efficacy of rVIII-SingleChain in preclinical tox species using thrombin generation.

The aim of this study was to evaluate the pharmacodynamic efficacy of rVIII-SingleChain in plasma of CD rats. The thrombin generation assay was selected as read-out for haemostatic efficacy, since it was shown to be a sensitive model discriminating pharmacological activity of FVIII products in a range of FVIII concentrations of 10-320 % of the norm.

rVIII-SingleChain was spiked at concentrations of 1 to 30 IU/mL based on chromogenic FVIII activity to plasma derived from female CD rats. Pharmacodynamic activity was determined based on a thrombin generation assay using a calibrated automated thrombogram. Coagulation was triggered using the PPP-Reagent. The TGA variables peak height, time to peak, lagtime, and endogenous thrombin potential were subjected to statistical analysis.

rVIII-SingleChain showed a concentration-dependent pharmacodynamics effect on thrombin generation compared to the negative control. Peak height increased and time to peak shortened concentration-dependently and statistically significantly with increasing concentrations of rVIII-SingleChain up to 10 IU/mL. There was no concentration-dependent effect of rVIII-SingleChain on lagtime, and no effect of rVIII-SingleChain on ETP.

rVIII-SingleChain showed a concentration-dependent pharmacodynamics effect on thrombin generation in plasma of CD rats, measured as an increase of thrombin peak and a reduction of time to peak,

supporting the rat as a pharmacological relevant animal model for non-clinical rVIII-SingleChain investigations.

Study: Pharmacodynamic efficacy of rVIII-SingleChain in monkey plasma using thrombin generation.

The aim of this study was to evaluate the pharmacodynamics efficacy of rVIII-SingleChain in plasma derived from cynomolgus monkeys.

The results demonstrated that rVIII-SingleChain showed a concentration-dependent pharmacodynamic effect in plasma derived from cynomolgus monkeys based on thrombin generation as characterized by an increase in thrombin peak and ETP and a reduction in time to peak supporting the cynomolgus monkey as pharmacological relevant animal model for non-clinical rVIII-SingleChain investigations.

***In vivo* studies**

Study: Pharmacodynamic comparison of rVIII-SingleChain and Advate regarding thromboelastography and thrombin generation parameters following a single intravenous injection to Haemophilia A mice.

The aim of this study was to compare the pharmacodynamic effect of rVIII-SingleChain and the marketed full length rFVIII product Advate on thromboelastography and thrombin generation parameters *ex vivo* in FVIII deficient whole blood (TEG/TEM) or plasma (TGA) using FVIII ko mice.

Both rFVIII products, rVIII-SingleChain and Advate, were administered intravenously at dose levels of 20 IU/kg according to chromogenic FVIII activity (in house measurements for rVIII-SingleChain, nominal activity for Advate) 15 min prior to blood sampling. Pharmacodynamic activity of the test substances was tested using thromboelastography/thromboelastometry to assess the viscoelasticity of whole blood and to monitor bleeding management in relation to blood clotting quality and thrombin generation in presence of Pathromtin SL and phospholipids (0.5 mM Phospholipid-TGT).

The pharmacodynamic activity of rVIII-SingleChain did not differ significantly from the marketed recombinant full-length FVIII product Advate and the overall hemodynamic capacity of rVIII-SingleChain appears to be comparable to Advate when dosed according to chromogenic FVIII activity.

Study: Pharmacodynamic comparison of rVIII-SingleChain and Advate regarding aPTT following a single intravenous injection to Haemophilia A mice.

The aim of this study was to compare the pharmacodynamic effect of rVIII-SingleChain and the marketed full length rFVIII product Advate in an aPTT *ex vivo* test using FVIII deficient plasma from FVIII ko mice. Additionally, FVIII activity was determined in the plasma samples using the chromogenic and the one-stage clotting FVIII assay to confirm exposure.

Both products were administered intravenously at a dose level of 20 IU/kg to FVIII ko mice 15 min prior to blood sampling. They were administered for the rVIII-SingleChain group according to chromogenic FVIII activity and for the Advate group according to nominal activity. FVIII activity (clotting and chromogen) was determined to confirm exposure, and activated partial thromboplastin time (aPTT) was analysed as a measure for pharmacodynamic activity (correction of hemostasis).

The results obtained for both rFVIII products for the activated partial thromboplastin time, the clotting FVIII activity, and the chromogenic FVIII activity compared to the control group demonstrated a clear effect on the endpoints measured. Both test items could restore the FVIII coagulation parameter aPTT to a comparable degree. At the same time, FVIII activity increased significantly in plasma, thereby confirming exposure. The increase in FVIII activity was slightly larger for rVIII-SingleChain as compared

to Advate in the chromogenic FVIII assay and smaller in the clotting FVIII assay indicating that changes in aPTT are in line with chromogenic FVIII activity, but correlate less stringent with clotting FVIII activity.

Studies: (A) Correction of hemostasis in FVIII ko mice following treatment with rVIII-SingleChain, Helixate, ReFacto AF and Humate P and (B): Correction of hemostasis in FVIII ko mice following treatment with rVIII-SingleChain, Helixate, ReFacto AF, Advate and Humate P.

The primary aim of these studies was to establish a dose-response relationship regarding the haemostatic efficacy of rVIII-SingleChain in haemophilia A mice (FVIII ko mice). In addition, effects of rVIII-SingleChain were compared with marketed, clinically relevant, rFVIII products, i.e. the full-length proteins Helixate and Advate, the B-domain-deleted protein ReFacto AF, and the plasma-derived human coagulation FVIII/von Willebrand Factor complex concentrate Humate P at dose levels between 1 IU/kg and 150 IU/kg FVIII activity, except for Humate P (41 IU/kg). As a secondary aim, aPTT was determined in all test groups at the end of the observation period.

The FVIII dose was determined by a chromogenic assay. It is noteworthy, that according to a one-stage clotting assay, the dose of rVIII-SingleChain was about 2.8 fold lower than that of Helixate, about 1.6 fold lower than that of ReFacto AF and about 2.3 fold lower than that of Advate.

The rFVIII concentrate rVIII-SingleChain demonstrated its haemostatic efficacy in haemophilia A mice. Thereby, the results obtained for the haemostatic efficacy differ slightly depending on the assay system used for dose adjustment chromogenic FVIII assay system vs. one-stage-clotting FVIII assay system).

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies have been performed (see discussion on non-clinical aspects).

Safety pharmacology programme

In accordance with the ICH guideline S6, the safety pharmacology investigation concerning measurements of cardiovascular variables was included in the 28 day repeat-dose toxicity study in monkeys. In addition, the assessment of potential neurobehavioral effects of rVIII-SingleChain was integrated in the subchronic repeat-dose toxicity study in rats. A further in depth investigation of potential effects of rVIII-SingleChain on hemodynamic and electrophysiological parameters was conducted in dogs and monkeys , whereas the impact on respiratory variables was assessed in dogs only. During these studies, rVIII-SingleChain was administered i.v. at a cumulative dose of up to 1550 IU/kg either under anaesthetized conditions or in conscious telemetered animals.

Study: rVIII-SingleChain: Toxicity study by intravenous bolus administration to CD rats for 4 weeks followed by a 14 day recovery period.

Potential effects of rVIII-SingleChain on the central nervous system were assessed as part of a repeat toxicity study in CD rats following intravenous (bolus) administration over a period of up to 4 weeks.

Overall, there were no treatment related changes to the clinical signs observed during the Irwin assessment. Furthermore, there were no macroscopic or histopathological changes observed which were considered to be indicative of an effect on the central nervous system.

Study: rVIII-SingleChain: Toxicity study by intravenous bolus administration to cynomolgus monkeys for 4 weeks.

The potential effects of rVIII-SingleChain on electrophysiological parameters and blood pressure were assessed as part of a repeat toxicity study performed in cynomolgus monkeys following daily intravenous (bolus) administration of rVIII-SingleChain over a period of 4 weeks, with a day 6 interim period.

Overall, the electrophysiology of the heart was considered unaffected by rVIII-SingleChain treatment. There were no obvious dose-related effects seen from the observations or the numerical data derived from the electrocardiograms recorded during the course of the study. Blood pressure and pulse rate measurements were considered to be unaffected by rVIII-SingleChain treatment.

Study: rVIII-SingleChain: Effects on general haemodynamics and respiratory variables in anaesthetized beagle dogs (intravenous infusion administration).

The potential effects of rVIII-SingleChain on electrophysiological and hemodynamic endpoints as well as respiratory parameters were assessed in anaesthetised dogs following a cumulative administration of increasing doses of rVIII-SingleChain. Two groups of dogs, each comprising 2 females and 2 males, received dose volumes of vehicle (0.9% w/v saline) equivalent to the volumes required to administer rVIII-SingleChain at dose levels of 50, 250 and 1250 IU/kg resulting in a cumulative dose of 1550 IU/kg rVIII-SingleChain. The test substances were administered at three consecutive intervals of 45 min duration comprising 30 min of test item administration followed by a period of 15 min between the end of one infusion to the start of the next infusion.

Overall, the pharmacologically relevant falls in cardiovascular parameters after treatment with rVIII-SingleChain (1250 IU/kg) were considered unlikely to be directly attributable to the active ingredient of rVIII-SingleChain. To confirm this hypothesis, follow-up studies were conducted in a conscious dog and monkeys under telemetric conditions

Study: Telemetric evaluation of cardiovascular effects in the conscious beagle dog (intravenous infusion administration).

The potential effects of rVIII-SingleChain on electrophysiological and hemodynamic parameters were assessed in a telemetered dog following a cumulative administration of increasing doses of rVIII-SingleChain. One telemetered, male dog received increasing dose volumes vehicle (dilution buffer for rVIII-SingleChain) equivalent to the volumes required to administer rVIII-SingleChain or doses of 50, 250 and 1250 IU/kg resulting in a cumulative dose of 1550 IU/kg rVIII-SingleChain. rVIII-SingleChain and the vehicle, dilution buffer for rVIII-SingleChain, were administered on separate days using the same dosing regimen as in the study performed in anaesthetized beagle dogs, thus test substances were administered at three consecutive intervals of 45 min duration. Endpoints assessed were similar to those investigated during the study performed in anaesthetized beagle dogs. Electrophysiological and hemodynamic variables were monitored for at least 90 min prior to the target time for commencement of dosing and continued for at least 20 h following the start of the first intravenous infusion on each of the two test sessions.

Following intravenous administration of the first two infusions of either dilution buffer for rVIII-SingleChain, or test item rVIII-SingleChain containing active FVIII, when applied at dose levels of 50 and 250 IU/kg, some differences were noted in cardiovascular parameters, notably for blood pressure and LVP parameters. Since recorded values were generally lower following rVIII-SingleChain administration the results were therefore not considered to be attributable to treatment with the active ingredient of rVIII-SingleChain.

Similarly to observations made in the study performed in anaesthetized beagle dogs, following intravenous administration of the third infusions of either dilution buffer for rVIII-SingleChain or rVIII-SingleChain at the dose level of 1250 IU/kg, changes were observed in arterial blood pressure (systolic, diastolic and mean), PR and QT intervals, heart rate, LVSP, LVEDP, dP/dtmax and dP/dtmin. These changes were of a similar magnitude and duration after infusion of both, dilution buffer for rVIII-SingleChain and rVIII-SingleChain (1250 IU/kg), and were therefore not considered to be attributable to treatment with the active ingredient of rVIII-SingleChain.

No effects were observed on the ECG (lead II) waveform morphology after treatment with either dilution buffer for rVIII-SingleChain or rVIII-SingleChain, throughout the study period.

During the first two infusions of either dilution buffer for rVIII-SingleChain or rVIII-SingleChain at dose levels of 50 and 250 IU/kg no adverse clinical signs were observed in the dog. However, during the third infusion of dilution buffer for rVIII-SingleChain, behavioural and clinical signs resembling a pseudo allergic reaction such as swelling and reddening of the extremities and the face, and pale gums were observed, which necessitated the halting of the third and final infusion after 23 min for reasons of animal welfare.

Alike, during the third infusion of rVIII-SingleChain (1250 IU/kg), clinical signs of a pseudoallergic reaction such as swelling around the eyes, face, neck, ears and forelimbs and reddening of the skin) were observed at a comparable time during the infusion (approximately +20 min), but the infusion continued until its scheduled completion after veterinary advice, and since the apparent effects were reversible as observed during the previous test session when investigating dilution buffer for rVIII-SingleChain.

The coincidence of timing and duration of the clinical signs observed after treatment with both dilution buffer for rVIII-SingleChain and rVIII-SingleChain strongly suggests that the observed effects were not directly related to treatment with the test item rVIII-SingleChain, but are related to a component of the dilution buffer for rVIII-SingleChain, which was common in the applied test items during both test sessions.

Taking into account the results obtained from the former study using anaesthetized dogs as well as the behavioural effects seen in this study using telemetered dogs, observed effects are believed to be due to the excipient PS80 of the rVIII-SingleChain dilution buffer for which dogs represent a notably sensitive species. Therefore, observed effects were not considered to be directly related to treatment with the active ingredient, the test item rVIII-SingleChain per se. The assumption of the PS80-induced cardiovascular and behavioural effects in the particularly sensitive species dog is supported by results obtained in the subsequent safety pharmacology study using telemetered monkeys, mimicking the dosing regimen of the two former studies in anaesthetized and telemetered beagle dogs as well as the subchronic toxicity study in cynomolgus monkeys. The lack of any treatment related clinical signs and cardiovascular or behavioural effects found in studies using cynomolgus monkeys, indicates that the dog is likely to be the more sensitive species to PS80, compared to the monkey. Thus, observed effects were not considered to be directly attributable to treatment with rVIII-SingleChain.

Study: Telemetric evaluation of cardiovascular effects in the conscious cynomolgus monkey (intravenous infusion administration).

The potential effects of rVIII-SingleChain on electrophysiological and hemodynamic parameters were assessed in telemetered cynomolgus monkeys following a cumulative administration of increasing doses of rVIII-SingleChain.

The observed differences in the PR-interval, heart rate and corresponding blood pressure were mild, rare and temporary. In addition, observed changes between the placebo control and rVIII-SingleChain or

dilution buffer for rVIII-SingleChain were small, dose-independent and sporadic and were therefore not considered to be directly related to treatment with rVIII-SingleChain. No adverse clinical signs or behavioural effects were observed during this study that were considered to be directly attributable to treatment with rVIII-SingleChain.

Pharmacodynamic drug interactions

No pharmacodynamic drug interactions have been performed.

2.3.3. Pharmacokinetics

Three PK studies after single i.v. dosing of rVIII-SingleChain were performed in monkeys and in FVIII ko mice (Table 2). Since i.v. administration is the only route available for administration to men, no other routes were tested preclinically. Further kinetic analysis was performed as part of the single and repeated dose toxicity studies.

Table 2: Overview of pharmacokinetic studies

Type of Study	Species and Strain	Method of Administration
Absorption	-	-
Plasma pharmacokinetics	Mouse /	i.v.
Plasma pharmaco-kinetics	Monkey / Cynomolgus/ Macaca fascicularis	i.v.
Plasma pharmacokinetics	Monkey / Cynomolgus/ Macaca fascicularis	i.v.
Distribution	-	-
Metabolism	-	-
Excretion	-	-
Pharmacokinetic drug interaction	-	-
Other pharmacokinetic studies	-	-

Methods of analysis

For measuring FVIII activity in plasma, two different test principles were used, one using a chromogenic assay (ChS) and one a one-stage (OS) clotting assay.

Absorption

Study: rVIII-SingleChain: PK study and tool antibody production in cynomolgus monkeys

The objective of this study was to assess the pharmacokinetics and dose proportionality of rVIII-SingleChain following a single intravenous dose at levels of 50 IU/kg and 250 IU/kg (Phase A). In addition, the systemic exposure following i.v. administration of rVIII-SingleChain was compared to that of marketed recombinant human FVIII products, i.e. Helixate and ReFacto AF. The objective of Phase B was – after the initial intravenous prime - to elicit a humoral immune response following repeated subcutaneous administration in combination with an adjuvant, for the purpose of harvesting species specific anti-product antibodies as an analytical tool for subsequent pivotal GLP-toxicity studies. Furthermore, appearance of an immune response against rVIII-SingleChain compared to ReFacto AF and Helixate, respectively, was investigated.

During the study, clinical condition, body weight, haematology, blood chemistry, bioanalytical, organ weight and macroscopic pathology investigations were undertaken.

During the phase A, rVIII-SingleChain displayed similar pharmacokinetic properties when compared with those of ReFacto AF but exhibited a slightly lower clearance when compared to Helixate. In addition after comparison of the 50 IU/kg dose groups with those of the 250 IU/kg dose groups the results show that for both rVIII-SingleChain and Helixate approximate dose proportionality holds in particular for those parameters that do not involve extrapolation, i.e. $C_{max,incr,obs}$, $C_{max,incr}$ and AUC_{24h}.

In terms of safety it could be concluded that single intravenous administrations of rVIII-SingleChain or the other FVIII concentrates (Helixate or ReFacto AF) at a dosage of 250 IU/kg to cynomolgus monkeys was well tolerated and did not exhibit any adverse effects of treatment with rVIII-SingleChain.

During Phase B, active immunization after biweekly repeated subcutaneous administration of rVIII-SingleChain, Helixate or ReFacto AF, when administered in combination with an adjuvant at a dosage of 350 IU/kg, elicited a fulminant anti-FVIII-antibody immune response in all animals but was not well tolerated. Under conditions of an active immunization, repeated treatment with heterologous human FVIII products resulted in a strong neutralizing anti-FVIII antibody response, which was significantly cross reactive with, and hence inhibitory to endogenous FVIII and elicited haemophilia A like symptoms to a greater or lesser degree in all animals treated with rVIII-SingleChain, Helixate or ReFacto AF.

Study: rVIII-SingleChain: PK study in cynomolgus monkeys

The objective of this study was to assess the pharmacokinetics of rVIII-SingleChain following a single intravenous dose of 250 IU/kg. In addition, the systemic exposure following i.v. administration of rVIII-SingleChain was compared to that of marketed recombinant human FVIII product, i.e. Advate. Prior to plasma sample analysis venous blood samples were collected from all animals at the following schedule: pre-dose, 0.25, 0.5, 1, 2, 4, 6, 12 and 24 h post dose.

Following a single intravenous bolus injection, rVIII-SingleChain and the comparator formulation Advate were well tolerated at a dosage of 250 IU/kg, with no clinical signs or reactions to treatment observed, nor any injection site observations considered associated with treatment.

Individual FVIII activities were measured using the chromogenic substrate assay. Corresponding FVIII plasma levels were calculated using standard human plasma as calibrator, which is calibrated from the manufacturer against WHO standard material. 100% of norm corresponds to 1 IU/mL FVIII. Resulting values were above baseline, following administration of rVIII-SingleChain and Advate up to 24 h post dose in all animals. Generally, for each of the formulations there were no notable differences in the pharmacokinetics of FVIII between sexes.

Following administration of rVIII-SingleChain, the maximum activity of FVIII was similar to that following administration of Advate.

Plasma levels of FVIII activity declined from a maximum at the first sampling time in an apparent bi-exponential manner with a terminal half-life ranging between 10-15 h for rVIII-SingleChain and ranging between 4.6-4.7 h for Advate.

The mean C_0 value following administration of rVIII-SingleChain was similar to that following administration of Advate. However, the mean AUC_t value (% of the norm*h/mL) following administration of rVIII-SingleChain was 1.7-fold higher than that after administration of Advate. When comparing exposure extrapolated to infinity (AUC), FVIII activity was approximately 2.3 fold greater following administration of rVIII-SingleChain to that following administration of Advate.

Accordingly, the CL value (IU/%*h) following administration of rVIII-SingleChain was approximately 2.2-fold higher compared to Advate. The mean terminal half-life following administration of rVIII-SingleChain was approximately 2.7-fold longer than that after administration of Advate.

Study: Pharmacokinetic evaluation of rFVIII/rVIII-SingleChain, Helixate, ReFacto AF and Advate in FVIII ko mice

The aim of this study was to establish a comparative pharmacokinetic analysis of rVIII-SingleChain in haemophilia A mice (FVIII ko mice) and thereby compare the profile head-to-head with other clinically relevant, recombinant human FVIII products, i.e. the full length protein Helixate and Advate and the B-domain-deleted protein ReFacto AF.

All test articles were injected intravenously at a clinically relevant dose of 100 IU/kg adjusted according to the one-stage clotting system activity of the four different test items. The subsequent determination of FVIII activity in plasma was performed by the chromogenic assay as well as the one-stage clotting system at different time points after administration of the four different test items.

The overall pharmacokinetic properties of rVIII-SingleChain did not differ largely from the other marketed recombinant human FVIII products Helixate, ReFacto AF or Advate when keeping the analytical system the same by adjusting the applied dose and measuring the respective FVIII:C plasma levels with either the chromogenic system or the one-stage clotting system. Hence this particular set up may reflect and predict the human pharmacokinetics and in turn the haemostatic efficacy in haemophilia A individuals most appropriately. The favourable PK profile of rVIII-SingleChain compared to other recombinant FVIII concentrates in Haemophilia A mice is consistent with the observations obtained in a cynomolgus monkey study when administering rVIII-SingleChain at doses of 50 IU/kg and 250 IU/kg.

Distribution

Distribution studies were not submitted (see discussion on non-clinical aspects).

Metabolism

Metabolism studies were not submitted (see discussion on non-clinical aspects).

Excretion

No separate elimination or excretion studies were submitted (see discussion on non-clinical aspects).

Pharmacokinetic drug interactions

No nonclinical drug interaction studies were performed.

Other pharmacokinetic studies

No other PK studies have been performed.

2.3.4. Toxicology

The i.v. single dose toxicity of rVIII-SingleChain was evaluated in rats and monkeys using doses of up to 1500 IU/kg.

Repeat-dose toxicity of rVIII-SingleChain was evaluated in rats and monkeys. In rats rVIII-SingleChain was administered intravenously at doses of 50, 250 and 1250 IU/kg on 28 consecutive days followed by a 14 day recovery phase. In monkeys rVIII-SingleChain was administered intravenously at doses of 50, 150 and 500 IU/kg on 28 consecutive days.

Local tolerance investigations were included in the single-dose and repeat-dose toxicity studies. Furthermore, a separate local tolerance study was performed in rabbits assessing potential effects of rVIII-SingleChain if applied according to the intended clinical route as well as in case an aberrant administration would occur.

The potential prothrombotic risk of rVIII-SingleChain was addressed in the venous-stasis-induced thrombosis model (modified Wessler test).

Table 3: Overview of toxicology studies

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (IU/kg BW)	GLP Compliance
Single-dose toxicity	Rat/	Bolus i.v.	Single dose	50, 250, and 1500	Yes
	Monkey/ Macaca fascicularis	Bolus i.v.	Single dose	50, 250, and 1500	Yes
Repeat-dose toxicity	Rat/	Bolus i.v.	28 days	50, 250 and 1250	Yes
	Monkey/ Macaca fascicularis	Bolus i.v.	28 days	50, 150 and 500	Yes
Genotoxicity	-	-	-	-	-
Carcinogenicity	-	-	-	-	-
Reproductive and developmental toxicity	-	-	-	-	-
Local Tolerance	Rabbit/ NZW	i.v. i.a. p.v.	Single dose	359 i.v. (= 1 mL/animal) 359 i.a. (=1 mL/animal 71.8 p.v. (= 0.2 mL/animal)	Yes
Other toxicity: Thrombogenicity	Rabbit/ NZW	i.v.	Single dose	150 IU/kg, 300 IU/kg, 500 IU/kg 1000 IU/kg	Yes

Single dose toxicity

Study: rVIII-SingleChain: Single dose toxicity study by intravenous bolus administration to rats.

The systemic toxic potential and toxicokinetics of rVIII-SingleChain was assessed over a 5 day period following a single dose by intravenous (bolus) administration in CD rats. Three groups, each comprising five male and five female rats received rVIII-SingleChain at doses of 50, 250 or 1500 IU/kg. A similarly constituted control group received isotonic saline (0.9 %) at the same volume-dose as the highest treated dose group (3.61 mL/kg). A further 3 males and 3 females were allocated to the control group and 9 males and 9 females were allocated to each treatment group (n=3 per group). These animals were used for toxicokinetic evaluation. During the study, clinical condition, mortality, body weight, food consumption, haematology, blood chemistry, toxicokinetics, urinalysis, organ weight, macropathology

and histopathology investigations were undertaken. Blood samples for toxicokinetic evaluation were taken at pre-dose, 0.25, 0.5, 1, 4, 6, 12 and 24 hours.

A single intravenous (bolus) injection of rVIII-SingleChain at doses up to 1500 IU/kg was well tolerated in the rat with no findings indicative of adverse toxicity and no irritation at the site of injection. Therefore, under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) is considered to be 1500 IU/kg.

Study: rVIII-SingleChain: Single dose toxicity study by intravenous bolus administration to cynomolgus monkeys.

The systemic toxic potential and toxicokinetics of rVIII-SingleChain was also assessed in a single dose study by intravenous (bolus) administration in cynomolgus monkeys. Three groups of 3 male and 3 female monkeys received a single dose of rVIII-SingleChain at 50, 250 or 1500 IU/kg on study day 1. A similarly constituted control group received isotonic saline (0.9 %) at the same frequency. Two male and two female animals from each group were killed on day 6 of the study, and the remaining 1 male and 1 female from each group were killed on day 11.

A single intravenous bolus injection of rVIII-SingleChain at doses up to 1500 IU/kg was well tolerated in cynomolgus monkeys with no effects observed. Under the conditions of this study, the NOAEL was considered to be 1500 IU/kg.

Repeat dose toxicity

Study: rVIII-SingleChain: Toxicity study by intravenous bolus administration to CD rats for 4 weeks followed by a 14 day recovery period.

The systemic toxic potential of rVIII-SingleChain to CD rats by intravenous (bolus) administration including toxicokinetics was assessed over a period of up to 4 weeks. The potential for any treatment-related effects to show recovery was assessed in a subsequent 2-week recovery period in selected animals. Five male and five female animals from each group were killed on day 6 as part of the interim kill to investigate initial toxicity before the development of any potential immune response against the heterologous human protein.

The administration of rVIII-SingleChain by intravenous (bolus) injection at doses up to 1250 IU/kg/day for 6 or 28 days was well tolerated in the rat with no findings indicative of adverse toxicity. An immune response resulting in the formation of antibodies against rVIII-SingleChain was apparent within the rVIII-SingleChain treated groups after 16 and 28 days of treatment, but there are no indications that this prevented an assessment of the toxicity of rVIII-SingleChain. Under the conditions of this study, the NOAEL is considered to be 1250 IU/kg.

Study: rVIII-SingleChain: Toxicity study by intravenous bolus administration to cynomolgus monkeys for 4 weeks.

The systemic toxic potential and toxicokinetics of rVIII-SingleChain to cynomolgus monkeys by intravenous (bolus) administration was also assessed over a period of 4 weeks, with a day 6 interim period. Three groups of 3 male and 3 female monkeys received rVIII-SingleChain at doses of 50, 150 or 500 IU/kg/day for 4 weeks. A similarly constituted control group received isotonic saline (0.9 %) with the same frequency as the treated groups but at a dose volume similar to the high dose group. A further one male and one female monkey were assigned to each group; these animals were dosed for 5 days and were killed on day 6 to investigate initial toxicity before the onset of any potential immune response against the heterologous human protein. During the study, clinical condition, mortality, bodyweight, ophthalmic examination, electrocardiography, blood pressure, haematology, blood chemistry,

toxicokinetics, antibody analysis, urinalysis, organ weight, macropathology and histopathology investigations were undertaken.

Administration of rVIII-SingleChain by intravenous (bolus) injection at doses up to 500 IU/kg was well tolerated in the cynomolgus monkey. An immune response resulting in the formation of antibodies against rVIII-SingleChain was apparent within the rVIII-SingleChain treated groups after 13 and 28 days of treatment. Under the conditions of this study, the NOAEL is considered to be the highest dose tested, i.e. 500 IU/kg.

Genotoxicity

Genotoxicity studies were not submitted (see discussion on non-clinical aspects).

Carcinogenicity

Carcinogenicity studies were not submitted (see discussion on non-clinical aspects).

Reproduction Toxicity

Reproduction studies were not submitted (see discussion on non-clinical aspects). Nonetheless, macro- and histopathological investigations of male and female reproductive organs have been included in the single-dose and repeat-dose toxicity studies (rats and monkeys) with no findings indicative of adverse toxicity.

Local Tolerance

Study: rVIII-SingleChain: Local tolerance study in the rabbit following intravenous, intra-arterial or perivenous injection.

This study investigated the local tolerance of rVIII-SingleChain in rabbits when reconstituted in water for injection and administered once by intravenous, intra-arterial and perivenous route.

There was no sign of toxicity or ill health in any rabbit during the observation period and bodyweight gain was considered to have been unaffected by treatment.

Overall, intravenous, intra-arterial and perivenous injection of rVIII-SingleChain was well tolerated with no local or systemic sign of reaction to treatment. In-life, macropathological and histological findings were considered to be due to the administration procedure.

Other toxicity studies

Study: rVIII-SingleChain (rFVIII): *In vivo* thrombogenicity test in the rabbit (modified Wessler test as described by Giles, A.R. 1980).

To determine the risk of thrombogenicity of rVIII-SingleChain, a thrombosis model was used which is technologically based on the induction of a temporary venous stasis by ligation of an appropriate vein. Thrombosis incidence and thrombus dry weight were then used as parameters for evaluation and comparison. In the present thrombogenicity study, the potential pro-thrombogenic effects of rVIII-SingleChain were evaluated at doses of 150, 300, 500 and 1000 IU/kg, when given via the i.v. route to 6 rabbits (3 females / 3 males) per dose and group. Twelve rabbits (6 females / 6 males) received physiological saline and acted as placebo group (negative control). FEIBA NF 500 (factor eight bypassing activity) was included in the study to act as a positive control, since it contains activated and non-activated factors of the prothrombin complex, which are able to activate the blood clotting cascade at

multiple sites. Since FEIBA NF 500 lacks FVIII, ReFacto AF was included as an additional comparator, comprising a non-activated rFVIII molecule. As a primary endpoint, incidence of thrombosis in both jugular veins after venous stasis was assessed.

rVIII-SingleChain showed only a minimal prothrombotic potential at the highest administered dose of 1000 IU/kg with no statistically significant effects at the lower doses of 150, 300 or 500 IU/kg, leading to the designation of a NOAEL of 500 IU/kg.

2.3.5. Ecotoxicity / environmental risk assessment

Afstyla is a recombinant replacement protein of the naturally occurring coagulation factor VIII. It is catabolized during human metabolism and no active molecule is excreted by the patient. In accordance with the guideline CHMP/SWP/4447/00 (1), Afstyla as a protein is exempted from an environmental risk assessment since proteins are unlikely to result in a significant risk to the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

Concerning pharmacology one in-vivo pharmacodynamic study was performed in coagulation Factor VIII deficient mice (FVIII knockout mice). Here, the i.v. administration of rVIII-SingleChain, respectively, revealed a significant dose-dependent reduction of total blood loss, time to haemostasis and activated partial thromboplastin time (aPTT) compared to the control group. The effects were comparable to other FVIII products, i.e. Helixate, ReFacto and Advate.

Originally, safety pharmacology investigations in terms of cardiovascular variables were included in the 28 repeat-dose toxicity study in monkeys. Here, the electrophysiology of the heart as well as blood pressure and pulse rate measurements were considered unaffected by Afstyla treatment.

The investigation of potential neuro-behavioural effects of Afstyla was integrated in the 28 repeat-dose toxicity study in rats. There were no treatment related changes to the clinical signs observed during the Irwin assessment. Further, there were no macroscopic or histopathological changes observed which were considered to be indicative of an effect on the central nervous system.

A further in depth investigation of potential effects of Afstyla on hemodynamic and electrophysiological parameters was conducted in dogs and monkeys, whereas the potential impact on respiratory variables was assessed in dogs only.

Unexpectedly, during the study conducted in anaesthetized beagle dogs statistically significant and pharmacologically relevant falls in cardiovascular parameters, i.e. arterial blood pressure, LVSP, LVEDP, Cardiac Output and Stroke Volume, following intravenous administration of the third infusion of Afstyla at the highest dose level of 1250 IU/kg were recorded, which had not been observed following treatment with 0.9% w/v saline. However, in the study using treatment of telemetered monkeys with either 0.9% saline or dilution buffer for Afstyla, when used as placebo control and vehicle control, respectively in comparison with Afstyla per se revealed no treatment related clinical signs on any of the behavioural or cardiovascular variables at cumulative doses of 1550 IU/kg. As it is known that dogs are PS80 sensitive, the cardiovascular and respiratory effects observed in study in dogs and the behavioural effects seen in the subsequent study in telemetered dogs, were considered to be due to the excipient PS80 of the rVIII-SingleChain dilution buffer, and not directly related to the treatment with the active ingredient.

Based on the mechanism of action of rVIII-SingleChain, representing a human blood coagulation factor, no secondary pharmacodynamic effects were expected. There were also no indications for secondary

pharmacodynamic effects observed during primary pharmacodynamics, safety pharmacological or toxicological investigations. This was considered acceptable.

Pharmacokinetics

In monkeys, Afstyla displayed similar pharmacokinetic properties when compared with those of ReFacto AF but exhibited a slightly lower clearance when compared to Helixate. In addition after comparison of the 50 IU/kg dose groups with those of the 250 IU/kg dose groups the results show that for both Afstyla and Helixate approximate dose proportionality holds in particular for those parameters that do not involve extrapolation, i.e. $C_{max,incr,obs}$, $C_{max,incr}$ and AUC_{24h}. In a second study in monkeys PK of 250 IU/kg, Afstyla was compared to that of Advate. Here, the mean terminal half-life following administration of Afstyla was approximately 2.7-fold longer than that after administration of Advate.

In FVIII deficient mice the overall pharmacokinetic properties of Afstyla were slightly superior, but did not differ largely from the other marketed recombinant human FVIII concentrates Helixate, ReFacto AF or Advate when adjusting the applied dose of Afstyla and measuring the respective FVIII:C plasma levels by the identical analytical test system, either the chromogenic system or the one-stage clotting system, and comparator properties were analysed according to their labelled potency dose.

Overall, the pharmacokinetic studies submitted are considered sufficient and appropriate to support marketing authorisation.

Toxicology

Single intravenous (bolus) injection of Afstyla at doses up to 1500 IU/kg was well tolerated in the rat with no findings indicative of adverse toxicity and no irritation at the site of injection. Similarly single intravenous bolus injection of Asftyla at doses up to 1500 IU/kg was well tolerated in cynomolgus monkeys with no effects observed. Accordingly, for both studies the NOAEL was considered to be 1500 IU/kg.

Repeat-dose toxicity of Afstyla was also evaluated in rats and monkeys. In rats the administration of Afstyla by intravenous (bolus) injection at doses of 50, 250 or 1250 IU/kg/day for 6 or 28 days was well tolerated with no findings indicative of adverse toxicity. An immune response resulting in the formation of antibodies against Afstyla was apparent within the Afstyla treated groups after 16 and 28 days of treatment, but there are no indications that this prevented an assessment of the toxicity of Afstyla. Under the conditions of this study, the NOAEL was considered to be 1250 IU/kg.

In monkeys Afstyla was administered intravenously at doses of 50, 150 and 500 IU/kg on 28 consecutive days. Administration of Afstyla by intravenous (bolus) injection at doses up to 500 IU/kg was well tolerated. An immune response resulting in the formation of antibodies against Afstyla was apparent within the Afstyla treated groups after 13 and 28 days of treatment. Under the conditions of this study, the NOAEL was considered to be the highest dose tested, i.e. 500 IU/kg.

Genotoxicity studies were not submitted as the active components of rVIII-SingleChain are recombinant counterparts of naturally occurring human plasma proteins. As mutagenic effects of FVIII were not expected, since there is no direct interaction with DNA to anticipate damage of DNA or interaction with DNA binding proteins, no studies with regard to the mutagenic potential of rVIII-SingleChain have been performed.

Carcinogenicity studies were not submitted in accordance with ICH S6 R1 and as the active component of rVIII-SingleChain is a recombinant counterpart of naturally occurring human plasma proteins, carcinogenicity studies are not regarded to be required and have not been performed.

A local tolerance study was performed in rabbits. Here intravenous, intra-arterial and perivenous injection of Afstyla was well tolerated with no local or systemic sign of reaction to treatment. Macro-pathological and histological findings were considered to be due to the administration procedure. Furthermore, local tolerance investigations were included in the single dose and repeat-dose toxicity studies (in rats and monkeys) showing good tolerability of Afstyla following i.v. administration.

A thrombogenicity study was conducted to evaluate the prothrombotic potential of Afstyla using the modified Wessler Test (as described by Giles, 1980). Here, Afstyla showed only a minimal prothrombotic potential at the highest administered dose of 1000 IU/kg with no statistically significant effects at the lower doses of 150, 300 or 500 IU/kg, leading to the designation of a NOAEL of 500 IU/kg.

Afstyla is a recombinant replacement protein of the naturally occurring coagulation factor VIII. It is catabolized during human metabolism and no active molecule is excreted by the patient. In accordance with the guideline CHMP/SWP/4447/00 (1), Afstyla as a protein is exempted from an environmental risk assessment since proteins are unlikely to result in a significant risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

Non-clinical data submitted as part of this application reveal no special hazard for humans based on conventional studies of safety pharmacology, single and repeated dose toxicity studies, local tolerability and thrombogenicity assessments.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

A GCP inspection at two clinical sites (Thailand and Malaysia) and the sponsor site (Germany) between 29 March 2016 and 20 May 2016. The outcome of the inspection carried out was issued on 04 July 2016. The Inspectors' recommendations on the GCP inspection are deemed to have no impact on the overall benefit/risk profile of the product.

- Tabular overview of clinical studies

Overview of rVIII-SingleChain Clinical Studies Contributing to the Clinical Development Program

Study, Status	Type of Study	Study Design	Primary objective(s) of the study	Number and Age of Subjects	Duration of Treatment	Location of study centers
Study 1001 Complete	Safety, Efficacy and PK	Phase I / III, prospective multicenter, open label with surgery substudy	Characterize the PK profile of rVIII-SingleChain Demonstrate efficacy in prevention and treatment of bleeding episodes Demonstrate efficacy of a routine prophylaxis regimen over on demand regimen Demonstrate efficacy of rVIII-SingleChain in surgical prophylaxis	174 subjects [a] *Surgery substudy*: 13 subjects Median (min, max) age: 31.3 (12, 64) years	Mean: 8.5 months (Actual) Median number of EDs: 64 EDs	Australia, Austria, Canada, Czech Republic, Germany, Hungary, Italy, Japan, Lebanon, Malaysia, Netherlands, Philippines, Poland, Romania, Russian Federation, South Africa, Spain, Ukraine, United Kingdom, United States

			Characterize the rate of inhibitor formation			
Study 3002 Complete	Safety, Efficacy and PK	Phase III, prospective multicenter, open label	Evaluate efficacy of rVIII-SingleChain in treatment of major and minor bleeding episodes based on investigator ' s 4-point assessment scale	84 subjects [a] 7.0 (1, 11) years 0 to < 6years: 35 subjects (thereof 20 subjects with PK data) ⩾ 6 to <12 years: 49 subjects (thereof 19 subjects with PK data)	Mean: 6.0 months (Actual) Median number of EDs: 0 to < 6years: 52 EDs ⩾ 6 to <12 years: 59 EDs	Australia, Austria, France, Georgia, Germany, Italy, Lebanon, Malaysia, Netherlands, Philippines, Poland, Portugal, Romania, Spain, Switzerland, Thailand, Turkey, Ukraine, United States
Study 3001 Ongoing	Safety, Efficacy	Phase III, prospective, multicenter, open label (extension study)	Evaluate safety of long-term use of rVIII-SingleChain	154 subjects [a, b] 27.6 (4, 65) years 0 to < 6years: 7 subjects ⩾ 6 to <12 years: 15 subjects ⩾ 12 to <18 years: 14 subjects ⩾ 18 to ⩽65 years: 118 subjects	⩾ 200 subjects achieving ⩾ 100 EDs (Planned)	Australia, Austria, Canada, Czech Republic, Georgia, Germany, Hungary, Italy, Japan, Lebanon, Malaysia, Netherlands, Philippines, Poland, Romania, South Africa, Spain, Thailand, Ukraine, United Kingdom, United States

Abbreviations: ED, exposure day; max, maximum; min, minimum; PK, Pharmacokinetics
[a] Safety population
[b] For Study 3001, data for subjects are included as of 29 May 2015.

2.4.2. Pharmacokinetics

The PK of rVIII-SingleChain was investigated throughout the clinical development program in studies 1001 and 3002.

- Study 1001: Pharmacokinetics in Subjects ≥ 12 to ≤ 65 Years of Age:

Study 1001 was an open-label, non-randomized, phase I / III study to assess the efficacy, safety, and PK of rVIII-SingleChain in subjects with severe haemophilia A for the prevention and treatment of bleeding episodes and for routine and surgical prophylaxis. The study consisted of 3 parts:

• Part 1 compared the single-dose PK of rVIII-SingleChain with Advate in 27 subjects ≥ 18 years

• Part 2 assessed the efficacy and safety of rVIII-SingleChain in subjects continuing from Part 1

• Part 3 assessed the safety, efficacy, PK of rVIII-SingleChain in 64 subjects ≥ 12 years and a repeat PK investigation in 30 of these 64 subjects, conducted 3 to 6 months after the initial investigation. Within Part 3 also a comparison of the PK of the low-strength and high-strength formulations of rVIII-SingleChain was performed.

- Study 3002: Pharmacokinetics in Subjects 0 to < 12 Years of Age:

Study 3002 was an open-label, multicenter, phase III study to assess the efficacy, safety, and PK of rVIII-SingleChain in subjects from 0 to < 12 years of age with severe haemophilia A. The study consists of a PK evaluation period, wherein PK data are assessed by a single dose of rVIII-SingleChain (50 IU/kg), and a treatment period, wherein subjects are treated with rVIII-SingleChain in an on-demand or prophylaxis regimen.

PK investigation of rVIII-SingleChain in 39 subjects 0 to < 12 years were performed (thereof 20 subjects 0 to < 6 years, 19 subjects ⩾ 6 to < 12 years). Furthermore a population PK analysis was conducted on the combined data from study 1001 and study 3002.

Analytical methods

Throughout the rVIII-SingleChain clinical development program, 8 assays were validated and employed to measure the following: FVIII activity measured with a one-stage clotting [OS] and chromogenic [ChS] assays, inhibitors against FVIII, non-inhibitory anti-drug antibodies (ADAs) (3 assays, including 1 screening and 2 confirmatory assays), and antibodies against CHO host cell proteins (2 assays, including 1 screening and 1 confirmatory assay).

Comparative Pharmacokinetics in Subjects ≥ 18 to ≤ 65 Years of Age

For the PK evaluation in Study 1001, Part 1, 27 subjects (≥ 18 to ≤ 65 years of age) first received a single injection of Advate (50 IU/kg) after a washout period of at least 4 days from any prior FVIII treatment. After another 4-day washout period after the Advate injection, the same subjects received a single injection of rVIII-SingleChain (50 IU/kg). The PK samples were collected before injection and at specific time points up to 72 h after injection of Advate and rVIII-SingleChain (time points: pre, 30 min, 1, 4, 8, 10, 24, 32, 48, 72h post-dose for both products).

Based on the dose-adjusted ChS assay data, mean values for AUC were higher, mean CL was lower, and mean t1/2 was longer with rVIII-SingleChain than Advate. The mean Cmax and IR values were similar between rVIII-SingleChain and Advate.

Table 4: Dose-adjusted FVIII Pharmacokinetic Parameters after Single Injections of rVIII-SingleChain and Advate for Subjects Dosed at 50 IU/kg (Non-compartmental Analysis; Study 1001, ChS Assay):

	Mean (CV%) Median (min, max)	
Parameter, unit	**Advate (N = 27)**	**rVIII-SingleChain (N = 27)**
IR[a], (IU/dL)/(IU/kg)	2.32 (16.4)	2.24 (15.9)
	2.29 (1.39, 2.87)	2.18 (1.47, 2.90)
C_{max}[a], IU/dL	116 (15.5)	113 (15.3)
	116 (69.7, 144)	109 (73.4, 147)
AUC_t, IU*h/dL	1490 (33.9)	2000 (29.1)
	1470 (685, 2550)	1960 (1110, 3390)
AUC_{inf}, IU*h/dL	1550 (35.5)	2090 (31.0)
	1510 (705, 2690)	2040 (1150, 3520)
CL, mL/h/kg	3.68 (38.2)	2.64 (32.1)
	3.34 (1.86, 7.10)	2.45 (1.40, 4.83)
V_{ss}, mL/kg	57.1 (19.7)	50.0 (15.0)
	54.3 (41.0, 94.7)	49.5 (32.4, 68.8)
$t_{1/2}$, h	13.3 (32.8)	14.5 (26.0)
	13.5 (7.19, 22.1)	13.6 (9.13, 23.9)
MRT, h	17.1 (32.5)	20.4 (26.9)
	16.8 (9.83, 31.1)	19.8 (12.8, 34.5)

Abbreviations: AUC_{inf}, area under the activity / concentration curve extrapolated to infinity; AUC_t, area under the activity / concentration curve to the last sample with quantifiable drug concentration; ChS, chromogenic substrate (assay); CV, coefficient of variation; CL, clearance; C_{max}, observed maximum plasma concentration; FVIII, factor VIII; IR, incremental recovery; IU, International Units; max, maximum; min, minimum; MRT, mean residence time; N, total number of subjects in the PK Population; rVIII-SingleChain, recombinant single-chain factor VIII; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady-state.

[a] For IR and C_{max}, predose correction was performed by subtracting each subject's FVIII activity level before dosing from the activity level obtained at each time point after dosing, as explained in Section 2.7.2.1.5.1. All other parameters are predose-uncorrected.

Non-compartmental PK analysis across all age groups

In the non-compartmental PK analysis across age groups, the PK parameters of subjects ≥ 12 to < 18 years and subjects ≥ 18 to ≤65 years were similar (table 5). Mean CL was higher in subjects 0 to < 12 years than in subjects ≥ 12 to ≤ 65 years (4.86 vs. 3.15 mL/h/kg, respectively), with consequently lower mean AUC and t1/2 values. The highest mean CL values and the lowest mean AUC values were observed in the youngest age group (subjects 0 to < 6 years).

Table 5: Summary of FVIII Pharmacokinetic Parameters Comparing Subjects by Age Group after Initial Injection of rVIII-SingleChain for Subjects Dosed at 50 IU/kg (Non-compartmental Analysis; PK Populations, Study 1001 and Study 3002, ChS Assay):

	Mean (CV%) Median (min, max)			
	Study 3002		Study 1001	
Parameter, unit	0 to < 6 years (n = 20)	≥ 6 to < 12 years (n = 19)	≥ 12 to < 18 years (n = 10)[a]	≥ 18 to ≤ 65 years (n = 81)[b]
IR[c, d], (IU/dL)/(IU/kg)	1.60 (21.1)	1.66 (19.7)	1.69 (24.8)	2.00 (20.8)
	1.55 (1.18, 2.76)	1.69 (0.920, 2.35)	1.76 (0.880, 2.44)	1.99 (0.868, 2.90)
C_{max}[c, d], IU/dL	80.2 (20.6)	83.5 (19.5)	89.7 (24.8)	106 (18.1)
	78.6 (59.3, 138)	84.5 (46.4, 117)	92.4 (45.5, 131)	106 (62.4, 151)
AUC_t, IU*h/dL	1010 (28.4)	1090 (26.4)	1480 (36.5)	1890 (32.0)
	945 (535, 1730)	1050 (626, 1640)	1470 (642, 2300)	1850 (895, 3840)
AUC_{inf}, IU*h/dL	1080 (31.0)	1170 (26.3)	1540 (36.5)	1960 (33.1)
	985 (561, 2010)	1120 (641, 1810)	1520 (683, 2380)	1910 (932, 4090)
CL, mL/h/kg	5.07 (29.6)	4.63 (29.5)	3.80 (46.9)	2.90 (34.4)
	5.08 (2.52, 8.92)	4.48 (2.79, 7.71)	3.31 (2.10, 7.32)	2.67 (1.26, 5.79)
V_{ss}, mL/kg	71.0 (11.8)	67.1 (22.3)	68.5 (29.9)	55.2 (20.8)
	70.7 (57.3, 88.3)	64.9 (44.3, 111)	62.0 (45.9, 121)	53.2 (32.4, 99.6)
$t_{1/2}$, h	10.4 (28.7)	10.2 (19.4)	14.3 (33.3)	14.2 (26.0)
	10.1 (5.19, 17.8)	10.0 (6.92, 14.8)	13.5 (6.32, 23.8)	13.7 (7.54, 23.9)
MRT, h	12.4 (25.0)	12.3 (16.8)	20.0 (32.2)	20.4 (25.8)
	13.0 (6.05, 17.9)	12.8 (8.22, 16.0)	18.6 (9.17, 31.7)	20.2 (10.8, 35.1)

Abbreviations: AUC_{inf}, area under the activity / concentration curve extrapolated to infinity; AUC_t, area under the activity / concentration curve to the last sample with quantifiable drug concentration; ChS, chromogenic substrate (assay); CL, clearance; C_{max}, observed maximum plasma concentration; CV, coefficient of variation; FVIII, factor VIII; IR, incremental recovery; IU, International Units; max, maximum; min, minimum; MRT, mean residence time; n, number of subjects within a given age group and study part; rVIII-SingleChain, recombinant single-chain factor VIII; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady-state.

[a] Number of subjects ≥ 12 to < 18 years in the Part 3 PK Population. (Part 1 only included subjects ≥ 18 to ≤ 65 years of age.)

[b] For IR and C_{max}, the number of subjects with available predose-corrected measurements was n = 80.

[c] For IR and C_{max}, predose correction was performed by subtracting each subject's FVIII activity level before dosing from the activity level obtained at each time point after dosing, as explained in Section 2.7.2.1.5.1. All other parameters are predose-uncorrected.

[d] In Study 3002, the first PK sample was taken 1 h after injection, vs. 10 to 15 min or 0.5 h after injection in Study 1001.

Population Pharmacokinetic Analysis

A total of 130 subjects from Studies 1001 and 3002 (age range of 0 to ≤ 65 years) contributed 1460 FVIII activity data points that were used in the population PK analysis of FVIII activity after IV injection of 50 IU/kg rVIII-SingleChain. A non-linear mixed effects model (NONMEM) was applied using NONMEM Version 7.2.

The PK for rVIII-SingleChain was described well by a 2-compartmental i.v. model of a combined additive and proportional residual error model, with central volume of distribution (V1), CL, peripheral volume of distribution (V2), and inter-compartmental clearance (Q) as disposition parameters.

Of all the covariates assessed (total body weight, age, body mass index, race, aspartate aminotransferase levels, alanine aminotransferase levels, creatinine clearance, baseline VWF levels, hematocrit, hepatitis positivity, the presence of ADAs, and geographical region), total body weight and baseline VWF levels had statistically significant influences on CL, and total body weight had a statistically significant influence on V1.

Model-based PK simulations of FVIII activity were performed for 20 to 50 IU/kg doses of rVIII-SingleChain after a single injection and after repeat injections every second day, every 3 days, 2 times weekly (on Day 0 and Day 3, or on Day 0 and Day 3.5), or 3 times weekly (on Day 0, Day 2, and Day 4, or on Day 0, Day 2, and Day 4.5) in subjects 0 to ≤ 65 years of age (table 6).

Table 6: Simulated Total FVIII Trough Activity after Repeat Injections of 20, 30, 40, and 50 IU/kg rVIII-SingleChain (ChS Assay)

	Total FVIII trough activity, IU/dL (median [90% PI])			
Simulated regimen (dosing days)	20 IU/kg	30 IU/kg	40 IU/kg	50 IU/kg
Every second day	3.2 [0.9, 10.4]	4.2 [1.1, 15.0]	5.3 [1.3, 19.6]	6.4 [1.4, 24.3]
Every 3 days	1.5 [0.5, 4.8]	1.8 [0.6, 6.5]	2.0 [0.6, 8.4]	2.3 [0.7, 10.2]
2 times weekly (Days 0 and 3)				
Day 3	1.5 [0.5, 4.6]	1.8 [0.6, 6.2]	2.0 [0.6, 7.9]	2.3 [0.6, 9.6]
Day 7	1.1 [0.4, 3.0]	1.2 [0.4, 3.7]	1.2 [0.4, 4.4]	1.3 [0.5, 5.2]
2 times weekly (Days 0 and 3.5)				
Day 3.5	1.2 [0.4, 3.6]	1.4 [0.5, 4.6]	1.5 [0.5, 5.8]	1.7 [0.5, 7.0]
Day 7	1.2 [0.4, 3.6]	1.4 [0.5, 4.6]	1.5 [0.5, 5.8]	1.7 [0.5, 7.0]
3 times weekly (Days 0, 2, and 4)				
Day 2	3.0 [0.9, 8.7]	4.0 [1.1, 12.5]	5.0 [1.3, 16.3]	6.1 [1.4, 20.1]
Day 4	3.1 [0.9, 9.9]	4.2 [1.1, 14.3]	5.3 [1.3, 18.7]	6.4 [1.4, 23.2]
Day 7	1.5 [0.5, 5.2]	1.8 [0.6, 7.2]	2.1 [0.6, 9.3]	2.3 [0.7, 11.3]
3 times weekly (Days 0, 2, and 4.5)				
Day 2	3.0 [0.9, 8.7]	4.0 [1.1, 12.5]	5.0 [1.3, 16.3]	6.1 [1.4, 20.1]
Day 4.5	2.1 [0.7, 7.0]	2.6 [0.8, 9.9]	3.1 [0.8, 12.9]	3.7 [0.9, 15.9]
Day 7	2.0 [0.7, 6.8]	2.5 [0.8, 9.6]	3.1 [0.8, 12.4]	3.6 [0.9, 15.3]

Abbreviations: ChS, chromogenic substrate (assay); FVIII, factor VIII; PI, prediction interval.

Dose proportionality and time dependency

Study 1001 compared the PK from the initial dose (64 subjects) and the repeated dose (30 subjects) over 3-6 months. No significant difference in PK profiles was observed between the initial and repeated doses.

Special populations

Impaired renal function:

Not Applicable.

Impaired hepatic function:

Not applicable.

Gender:

Not applicable.

Race:

In the covariate analysis of the population PK model, race (white, black or African American, Asian, other) was not found to have a statistically significant influence on CL or V1.

Elderly:

No subjects > 65 years were enrolled in any of the clinical studies. Therefore, no PK data in the elderly were generated.

Paediatrics:

The pharmacokinetics (PK) of rVIII-SingleChain in children (i.e. subjects 0 to <12 years of age) was evaluated within Study 3002. PK was assessed after a single dose of rFVIII-SingleChain (50 IU/kg). Blood samples were taken before injection and at specific sampling time points up to 48 h after injection (1, 5, 10, 24, 48 h).

The PK of Afstyla was evaluated in 10 previously treated adolescents (12 to <18 years of age) and 39 previously treated children (0 to <12 years of age) following an intravenous injection of a single dose of 50 IU/kg. All patients had been diagnosed with severe haemophilia A with <1% factor VIII.

The PK parameters presented here are based on plasma factor VIII activity measured by the chromogenic substrate assay.

As expected, differences in PK were seen between children (0 to < 12 years) in Study 3002 and adults/adolescents ≥ 12 to ≤ 65) in Study 1001.

Pharmacokinetic interaction studies

Neither in vitro Cytochrome P 450 hepatic drug metabolism nor drug-drug interaction studies were considered applicable for the in vitro investigation of rVIII-SingleChain. rVIII-SingleChain is a therapeutic protein metabolized by the same catabolic pathways as endogenous FVIII and results in the same amino acid fragments.

Special studies- immunogenicity

The PK findings corroborate those of the immunogenicity testing. No FVIII inhibitor development or antibodies against CHO host cell proteins were detected for any tested subject receiving rVIII-SingleChain. The 14 subjects with PK data (4 in Study 1001, 10 in Study 3002) who tested positive for non-inhibitory ADAs at any time did not have any relevant differences in their FVIII activity profiles or PK parameters compared to the 116 subjects with PK data who tested negative for ADAs.

2.4.3. Pharmacodynamics

No pharmacodynamics studies were conducted.

2.4.4. Discussion on clinical pharmacology

Chromogenic and one-stage clotting assay as used throughout all clinical studies have been validated. For both assay types, variance lies within the acceptable range.

A factor 1.8 [x] (x defined as OS FVIII activity) most closely aligns OS activity results with ChS activity results. The information of discrepancy between OS and ChS assay results and the use of a correction factor is given in posology section 4.2 of the SmPC. The conversion factor was changed from 1.8 to 2 in order to facilitate the use in clinical practice and to align with authorisation recommendations in other countries. Both conversion factors 1.8 and 2 are within the currently accepted range of variability in clinical practice and provide an equally acceptable value to monitor post-infusion plasma samples in patients treated with rVIII-SingleChain.Pharmacokinetics of Afstyla (lonoctocog alfa) was evaluated in a total of 130 previously treated male patients suffering from severe haemophilia A (<1%) in two pivotal studies, i.e. CSL627_1001 (adults and adolescents) and CSL627_3002 (children).

Absorption studies were not conducted since the route of administration is I.V.; no distribution studies were performed since Afstyla contains a recombinant endogenous FVIII protein and metabolism, elimination or excretion studies are not applicable since rFVIII is an endogenous protein that is catabolized via normal physiological pathways. No drug-drug interaction studies were conducted, since no drug interaction is expected given the close comparability of Afstyla to the native human FVIII. These omissions are considered to be acceptable.

In study 1001 part 1 PK of lonoctocog alfa was compared with PK of Advate in 27 patients. In brief, PK of rFVIII-Single Chain and Advate are comparable. rFVIII-Single Chain revealed slightly higher t½ and AUC values whereas the clearance was somewhat reduced in comparison to Advate. However, these differences are not considered clinically relevant. The applicant did not claim different posology recommendations but used the posology wording as suggested by the Core SPC, which is supported.

According to the guideline on the clinical investigation of recombinant factor VIII and IX products, the applicant compared initial and repeat PK. Based on the data provided it is agreed to the applicant that there are no obvious differences between initial and repeat PK.

As requested by the guideline on the clinical investigation of recombinant factor VIII and IX products, a PK comparison of high- and low-strength formulations of rVIII-SingleChain was performed. Furthermore, there was no clinically relevant effect of rVIII-SingleChain lot on the PK of FVIII following either single or repeat IV injections at a dose of 50 IU/kg.

In terms of paediatric patients PK data are available from 39 patients (20 in the age group 0 to <6 years and 19 in the age group ≥6 to <12 years). In adolescents i.e. ≥12 to <18 years, PK was evaluated in 10 patients. Overall, PK data in these age groups is considered sufficient.

No PK data in the elderly were generated as no subjects > 65 years were enrolled in any of the clinical studies. This is in line with the guideline on the clinical investigation of recombinant factor VIII and IX products.

Population PK simulations support the proposed prophylactic dosing regimen of 20 to 50 IU/kg rVIII-SingleChain 2 times weekly and 3 times weekly as the simulations predict that the majority of subjects, i.e. approximately 54% to 98% would maintain their trough total activity at >1% at all times. In the younger children (<6yrs) in the lowest dose groups 20 IU/kg 2 times weekly, less than 50% achieve FVIII trough levels >1%. Accordingly, the applicant proposed to increase the recommended starting dose from 20 to 50 IU/kg 2 to 3 times weekly to 30 to 50 IU/kg 2 to 3 times weekly for children <12yrs.

No specific pharmacodynamics studies were conducted as the PD effects of FVIII are closely associated with its PK parameters. This was considered acceptable.

2.4.5. Conclusions on clinical pharmacology

PK of rVIII-SingleChain has been thoroughly characterised in a sufficient number of patients across all age groups. Requirements for PK investigations as laid down in the Guideline on the clinical investigation of recombinant factor VIIIand IX products have been fulfilled. Furthermore, differences between results from OS and ChS assay have been addressed. Looking at ChS results, lonoctocog alfa does exert a PK profile comparable with other recombinant FVIII concentrates.

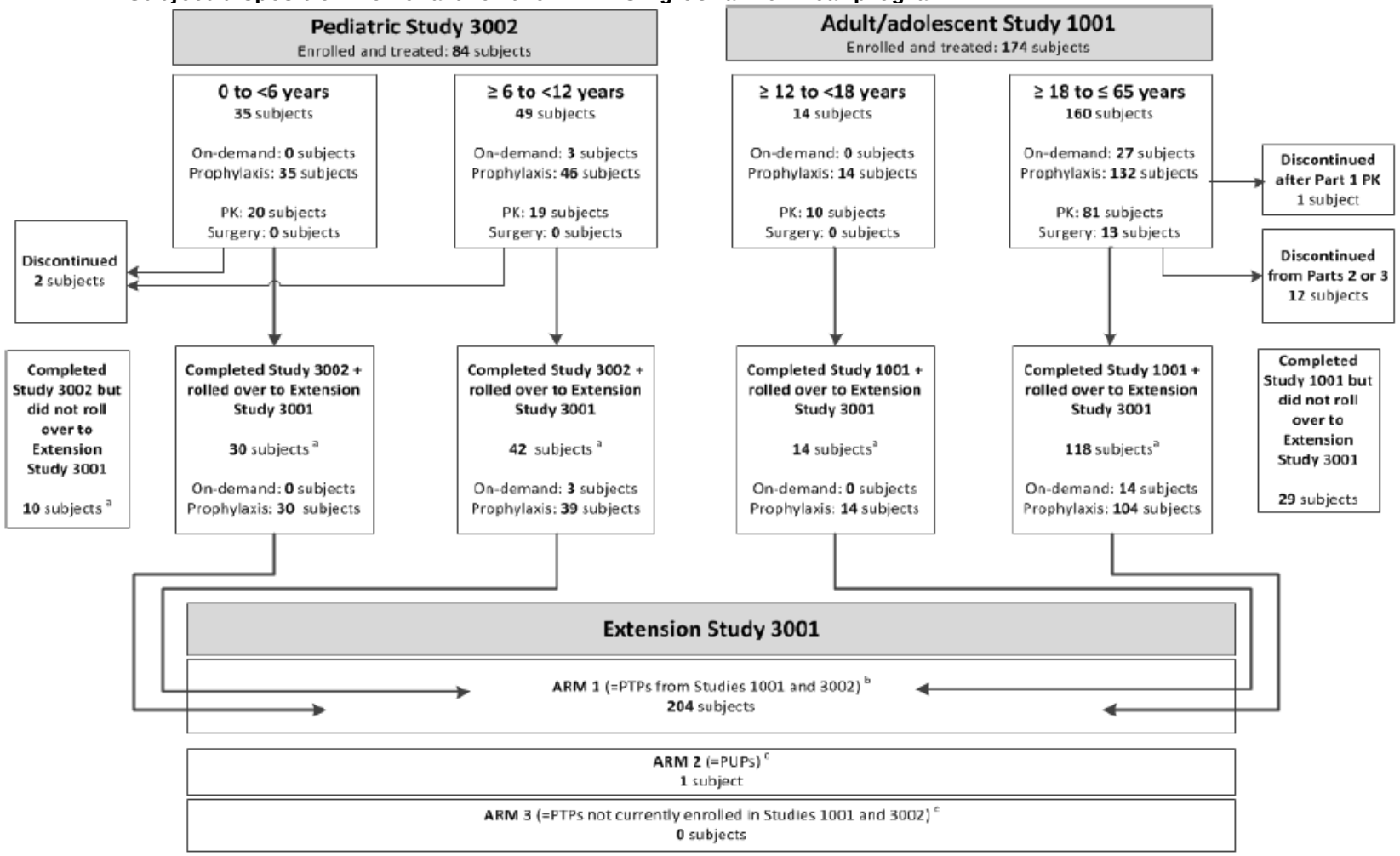
2.5. Clinical efficacy

The recombinant human coagulation factor VIII, single chain (rVIII-SingleChain) clinical development program includes two completed studies (CSL627_1001 and CSL627_3002) and one ongoing study (CSL627_3001) in subjects with severe haemophilia A.

2.5.1. Dose response and main clinical studies

The rVIII-SingleChain clinical program was designed to determine the PK profile, safety and efficacy of rVIII-SingleChain in adult and pediatric subjects with haemophilia A (FVIII activity < 1%) and consists of 3 studies (1001, 3002 and 3001).

Figure 3.2.1 Subject disposition flowchart for the rVIII-SingleChain clinical program



2.5.2. Dose response

Please see clinical pharmacology section where the treatment schedule is presented and discussed.

2.5.3. Main clinical studies

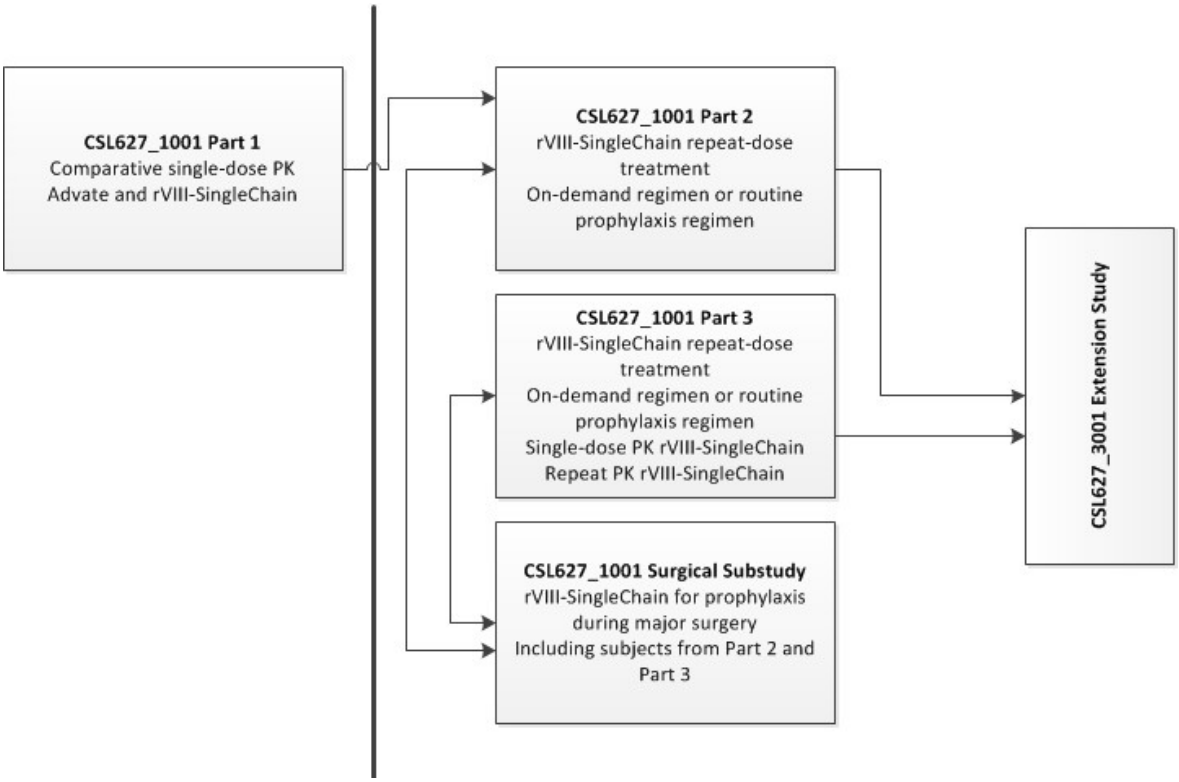
Study 1001: (Subjects ≥ 12 to ≤ 65 Years)

Study 1001 was an open-label, non-randomized, phase I / III study in male subjects ≥ 18 to ≤ 65 years of age (Part 1 and Part 2), and ≥ 12 to ≤ 65 years of age (Part 3) with severe haemophilia A (residual FVIII activity < 1%). To be enrolled in this study, subjects were required to have received a FVIII replacement product for > 150 EDs and have no history of FVIII inhibitors. The study consisted of 3 parts (Figure 3.4.1). Part 1 was a single-dose, crossover, PK comparison of Advate (50 IU/kg) and rVIII-SingleChain (50 IU/kg).

Part 2 assessed the efficacy and safety of on-demand and prophylaxis regimens with continued dosing of rVIII-SingleChain in subjects from Part 1. Part 3 assessed the safety and efficacy of on-demand and prophylaxis regimens with rVIII-SingleChain in newly enrolled subjects, and included initial and repeat PK assessments of rVIII-SingleChain in a subgroup of subjects. The study also included a surgical substudy for subjects enrolled in Parts 2 and 3.

Subjects in Parts 2 and 3 were to be treated until at least 104 subjects had reached 50 EDs to assess the risk of inhibitor formation as requested by the FDA. Thereafter, all subjects could roll over into Extension Study 3001.

Figure 3.4.1 Study 1001: Study Design



Methods

- **Study participants**

This study was performed as a multicenter study in the United States, Japan, Europe, and the rest of the world (ie, Australia, Canada, Lebanon, Malaysia, Philippines, Russian Federation, South Africa, and Ukraine).

Key inclusion criteria for Study 1001 included the following:

- Diagnosis of severe haemophilia A defined as < 1% FVIII:C documented in medical records.
- Males between ⩾ 18 and ⩽ 65 years of age (Parts 1 and 2).
- Males between ⩾ 12 and ⩽ 65 years of age (Part 3).
- Subjects who had received or were currently receiving FVIII products (plasma-derived and/or recombinant FVIII) and have had > 150 EDs with a FVIII product.
- Written informed consent for study participation had been obtained before undergoing any study specific procedures.

Key exclusion criteria for Study 1001 included the following:

- Any history of or current FVIII inhibitors
- Any first order family history of FVIII inhibitors
- Known hypersensitivity (allergic reaction or anaphylaxis) to any FVIII product or hamster protein.
- Any known congenital or acquired coagulation disorder other than congenital FVIII deficiency.
- Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months prior to Day 1.

- **Treatments**

Phase 1/3 Study in adults and adolescents ≥12 to ≤65 years of age (study 1001)

Study 1001 (pivotal adult / adolescent study, completed) was a phase I / III open-label, multicenter, crossover safety, efficacy, and pharmacokinetic (PK) study of rVIII-SingleChain compared to Advate in subjects with haemophilia A, and a repeat PK, safety, and efficacy study of rVIII-SingleChain. This study included a surgical substudy.

rVIII-SingleChain and Advate were administered as iv injections, with actual doses based on the subject's body weight.

Part 1

In Part 1 of the study, subjects were to receive a single iv dose of 50 IU/kg Advate on Day 1, after a 4-day wash-out period. Blood draws for the full PK analysis were to be performed up to 72 h after injection. Subjects would then wait 1 additional day to achieve a 4-day wash-out period before receiving a dose of rVIII-SingleChain at 50 IU/kg, followed by blood draws for the full PK analysis up to 72 h after injection.

At the conclusion of the 72 h PK blood collection for rVIII-SingleChain, subjects were to complete the follow-up visit and begin the on-demand or prophylaxis treatment period in Part 2.

Part 2

After subjects completed the PK follow-up assessment in Part 1, they were to start the on-demand or prophylaxis treatment period with rVIII-SingleChain. The first 5 subjects that continued into Part 2 were assigned to an on-demand regimen, to ensure that the haemostatic potential of rVIII-SingleChain was adequate based on treatment of bleeding episodes. After establishing the haemostatic potential, the remaining subjects from Part 1 continuing to Part 2 could be assigned to either an on-demand regimen or prophylaxis.

All prophylaxis subjects were to receive rVIII-SingleChain at a dose of 20 to 40 IU/kg body weight every other day or 20 to 50 IU/kg body weight 2 to 3 times per week, or at other doses and frequencies at the investigator's discretion.

All on-demand treatment subjects were to receive rVIII-SingleChain at a dose similar to the FVIII product used prior to enrollment for the same type of bleeding event, and continue in the study until 50 EDs or until at least 104 subjects reached 50 EDs.

Part 3

Part 3 was initiated following the collection and evaluation of the interim Part 1 PK analysis, which confirmed the acceptability of following the WFH dosing for the expanded cohort (Part 3). At least 13 new subjects were to participate in the full PK evaluation of rVIII-SingleChain and should have received a single dose of 50 IU/kg. Repeat PK analysis, using the same strength of rVIII-SingleChain, was to be performed after 3 to 6 months. After the initial PK, subjects were to then begin on-demand or prophylaxis treatment and continue treatment for at least 50 EDs or until at least 104 subjects reached 50 EDs.

Perioperative prophylaxis treatment (Parts 2 and 3)

For subjects entering this part of the study, the dose regimen of rVIII-SingleChain was to be individualized based on the type of surgery and the clinical status of the subject.

Prophylaxis treatment regimen

The investigator determined the rVIII-SingleChain prophylaxis dose and dosing schedule for the subject based upon the subject's PK profile (if available), rVIII-SingleChain PK profile, previous FVIII treatment regimen, bleeding phenotype (if available), and taking into consideration the World Federation of Haemophilia (WFH) guidelines [WFH, 2012]. In the previous pivotal studies, most subjects received prophylaxis treatment with an initial range of 20 to 50 IU/kg rVIII-SingleChain 2 to 3 times per week.

On-demand treatment of bleeding episodes

In the event of a bleeding episode, subjects were treated at a dose pre-determined by the investigator based on the type and severity of the bleeding episode. All subjects were to treat bleeding episodes with rVIII-SingleChain when they occurred, regardless of the assigned treatment regimen. The desired FVIII level for the treatment of a bleeding episode (on-demand treatment) was based on the recommendations of the WFH [WFH, 2012].

- **Objectives**

The primary objectives were to characterize the PK profile of rVIII-SingleChain, to demonstrate efficacy in the prevention and treatment of bleeding episodes, to demonstrate the efficacy of a routine prophylaxis regimen over an on-demand regimen, to demonstrate the efficacy of rVIII-SingleChain in surgical prophylaxis, and to characterize the rate of inhibitor formation.

The secondary objectives were to characterize the safety profile of rVIII-SingleChain and to compare the PK profile of rVIII-SingleChain to Advate.

- **Outcomes / endpoints**

The primary efficacy endpoints were:

- Control and prevention of bleeding episodes: rate of treatment success for bleeding episodes defined as a rating of "excellent" or "good" on the investigator's overall clinical assessment of haemostatic efficacy 4-point scale

  To estimate the rate of treatment success, the numerator included the number of bleeding episodes treated with rVIII-SingleChain and rated as "excellent" or "good", and the denominator included all treated bleeding episodes. In the primary analysis, treated bleeding episodes with missing investigator ratings were counted as treatment failures. In the 2 sensitivity analyses, bleeding episodes with missing investigator ratings were either excluded from the calculation, or counted as treatment successes.

- Routine prophylaxis: annualized spontaneous bleeding rate (AsBR), comparison of on-demand regimen to prophylaxis regimen

- Perioperative prophylaxis: rate of treatment success during the surgical substudy defined as an investigator rating of "excellent" or "good" on a 4-point efficacy evaluation of surgical treatment scale

The secondary and other efficacy endpoints were:

- Control and prevention of bleeding episodes: number of injections of rVIII-SingleChain required to achieve hemostasis, rate of treatment success for major bleeding episodes defined as a rating of "excellent" or "good" on the investigator's overall clinical assessment of haemostatic efficacy 4-point scale, and consumption of rVIII-SingleChain

- Routine prophylaxis: ABR and consumption of rVIII-SingleChain

- Perioperative prophylaxis: consumption of rVIII-SingleChain during surgical prophylaxis, predicted and estimated blood loss during surgery, predicted and actual transfusion requirements during surgery, change in hemoglobin levels between baseline, intra-operation and post-operation

The primary safety endpoint was the incidence of FVIII inhibitors associated with the use of rVIII-SingleChain. Safety was also assessed by adverse events (AEs), serious adverse events (SAEs), local tolerability at the site of injection, laboratory safety parameters, measurement of non-inhibitory anti-drug antibodies (ADAs) and antibodies against CHO host cell proteins, vital signs before and after injection, and physical examination.

- **Sample size**

Approximately 30 subjects were to be enrolled into Part 1 of the study, to ensure 26 evaluable subjects for the PK comparison (all subjects receiving comparator first, then Afstyla). Under the assumption that the expected ratio of means is 1, a coefficient of variation of 0.300, and an equivalence margin of 0.8 – 1.2, it was calculated that a TOST with alpha = 2.5% (1-sided per test) had about 80% power to confirm equivalence.

To ensure at least 104 subjects evaluable for inhibitor development, approximately 100 additional subjects were to be enrolled in part 3. It was assumed that at most 2 subjects out of 104 would develop an inhibitor during the study. This would maintain an upper 95%-CI within an acceptable upper bound of 6.8% (FDA requirement).

- **Randomisation**

n/a

- **Blinding (masking)**

n/a

- **Statistical methods**

Inhibitor development, haemostatic efficacy and bleeding events were key parameter of interest in study CLS627_1001.

Inhibitor formation was defined as any inhibitor (≥ 0.6 BU/mL) identified and confirmed by re-testing.

For estimating the inhibitor incidence, the numerator included all subjects with inhibitors regardless of EDs to rVIII-SingleChain; and the denominator included subjects with at least 50 EDs plus subjects with less than 50 EDs but with inhibitors. A 2-sided 95% exact CI for the incidence of inhibitor formation was calculated. If zero inhibitors were observed, then a 1-sided 97.5% upper confidence limit was calculated. SAS' FREQ procedure was applied to produce an exact CI, which used the Clopper-Pearson CI as the default method. Success was achieved if the upper confidence limit was less than the acceptable upper limit of 6.8%.

The rate of successfully treated bleeding episodes (i.e. investigator assessment of 'excellent / good') was calculated including the corresponding 95% CI. The 95%-CI was calculated applying a repeated measures model using generalized estimating equations and an independent correlation structure to account for within-subject correlation. The following treated bleeds were considered treatment failures: investigator assessment of moderate/ poor/none; treated with products other than rFVIII-SingleChain; having missing investigator ratings.

The AsBR defined by: 365.25*(number of spontaneous bleeding episodes) / (observed treatment period of interest).

(only spontaneous bleeding episodes requiring treatment outside the PK and surgical periods were included). The AsBR was presented for various subgroups by means of descriptive statistical characteristics. The primary comparison of interest for AsBR was the comparison between the prophylaxis arm and the on- demand arm. A Poisson regression model was used to test the null hypothesis of no difference regarding AsBR between both groups. The corresponding prophylaxis/on demand ratio with 95% CI was also presented. As a secondary analysis, the AsBR with prophylaxis treatment on study CSL627-1001 was compared to that of an on-demand/prevention historical control (data from Biostate study CSLCT-BIO-07-47); again applying poisson regression.

A hierarchical approach was used to deal with the multiplicity resulting from the assessment of the key parameter:

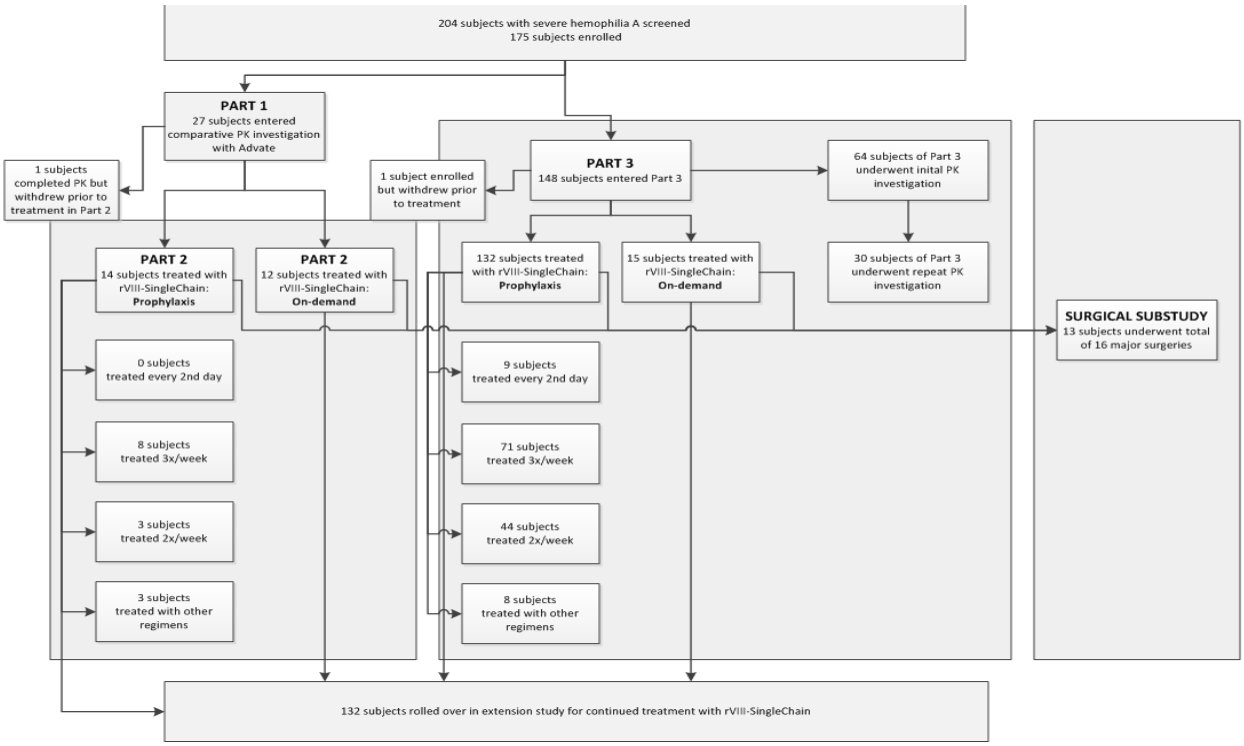
The procedure started with inhibitor development. If the upper limit of the 97.5%-CI for the risk of inhibitor development was greater than 6.8%, the study would have failed and further testing stopped. Otherwise testing proceeded to the evaluation of haemostatic efficacy for the treatment of bleeding

episodes. If the lower limit of the 95% CI for the observed rate of successfully treated bleeds would be less than 70%, then the study would have failed on this endpoint and further testing would be stopped. Otherwise, testing proceeded to the AsBR. If a test of the null hypothesis of no difference between the prophylaxis and the on-demand groups was not rejected at the two-sided 0.05 level, then the study would have failed on this endpoint and further testing stopped.

Results

- **Participant flow**

Figure 3.4.2 Disposition of Subjects (All Subjects, Study 1001)



Abbreviations: PK, pharmacokinetic(s); rVIII-SingleChain, recombinant single-chain factor VIII.

Source: Study 1001 CSR, Figure 10-1

Of the 204 subjects screened, 175 subjects were enrolled, 174 subjects comprised the Safety Population, and 173 subjects comprised the Efficacy Population. One subject was excluded from the Efficacy Population because he withdrew from the study before treatment with rVIII-SingleChain in Part 2.

Seventeen subjects (13 on the prophylaxis regimen and 4 using the on-demand regimen) were excluded from the PP Population due to lack of compliance with the prescribed dose or the prescribed prophylaxis regimen. The PP Population thus comprised 156 subjects.

The Surgical Population comprised 13 subjects (3 subjects in the on-demand group and 10 subjects in the prophylaxis group) who received at least 1 dose of rVIII-SingleChain during the surgical substudy (Table 7).

Table 7: Analysis Populations (Study 1001)

	Number of subjects
Screened	204
Enrolled	175
Safety Population	174
Efficacy Population	173
Prophylaxis	146
On-demand	27
Per protocol Population	156
Pharmacokinetics Population	91
Pharmacokinetics Population, Part 1	27
Pharmacokinetics Population, Part 3	64
Surgical Population	13

- **Recruitment**

Study 1001 enrolled 175 male PTPs ≥ 12 to ≤65 years (14 subjects ≥ 12 to <18 years; 161 subjects≥ to ≤ 65 years) with severe haemophilia A and > 150 previous EDs to FVIII prior to enrollment. The efficacy population of Study 1001 comprised 173 subjects ≥12 to ≤65 years (27 subjects on an on-demand regimen, 146 subjects on a prophylaxis regimen) exposed to rVIII-SingleChain for 14,306 EDs and treating 848 bleeding episodes overall. In the surgical substudy, 13 subjects received rVIII-SingleChain for a total of 16 surgical procedures, comprising major surgeries.

- **Conduct of the study**

During the course of study 1001, 4 global protocol amendments were implemented with the following main changes:

Amendment 1, dated 29 February 2012:

PK sample collection time in Part 1 changed from 28 to 32 hours post dose throughout protocol.

Amendment 2, dated 06 July 2012:

PK sample collection time points changed in Part 3; Statement of inclusion of Japanese centers in Part 3; Schedule of Assessment tables revised for clarity; IMP reconstitution table updated for multiple presentations and concentrations; Required number of evaluable subjects clarified; Exclusion of subjects not capable of home treatment added; Additional safety criteria added; Prior FVIII half-life and recovery collection specified for Part 3; Clarification of pharmacokinetic population

Amendment 3, dated 24 May 2013:

Duration of the subject study participation was clarified to allow subjects to be treated with Afstyla for 50 EDs and continue on treatment until end-of-study visit or extension study; Cohort screening size was increased to ensure sufficient evaluable subjects; Laboratory assessments and confirmation of results were clarified for central and local laboratories; The roles and responsibilities of the IDMC were updated to provide increased subject safety; Recording of actual dosing over nominal dosing was clarified to accurately reflect dosing; Definitions of overdose, treatment compliance, and retention of samples were

added to guide sites in proper study conduct; Assessment for antibodies against CHO cells was added for subject safety

Amendment 4, dated 10 March 2014:

Addition of a co-primary objective of efficacy of routine prophylaxis treatment over on-demand treatment; Addition of AsBR as a primary endpoint; Addition of annualized bleeding rate (ABR) as a secondary endpoint

- **Baseline data**

The subject population was all male and predominantly White (72.3% in the Efficacy Population). Subjects had a mean age of 31.3 years (minimum, maximum: 12, 64 years).

There were 14 subjects ≥ 12 to < 18 years of age, all of whom were enrolled in the prophylaxis group.

Subjects had a mean weight of 74.6 kg. The demographic characteristics were generally similar in the 2 treatment groups in the Efficacy Population.

Table 8: Demographic Characteristics of Adult / Adolescent Subjects by Treatment Regimen (Efficacy Population, Study 1001)

Characteristics	On-demand (N = 27)	Prophylaxis (N = 146)	Overall (N = 173)
Age, years			
n	27	146	173
Mean (SD)	40.3 (12.40)	29.7 (10.96)	31.3 (11.80)
Median	39.0	28.0	29.0
Min, max	23, 64	12, 58	12, 64
Age group, n (%)			
≥ 12 to < 18 years	0	14 (9.6)	14 (8.1)
≥ 18 to ≤ 65 years	27 (100.0)	132 (90.4)	159 (91.9)
Weight, kg			
Mean (SD)	78.1 (15.63)	74.0 (17.26)	74.6 (17.04)
Median	76.0	74.6	75.0
Min, max	39, 110	27, 120	27, 120
Race, n (%)			
Asian	1 (3.7)	30 (20.5)	31 (17.9)
Black or African American	3 (11.1)	11 (7.5)	14 (8.1)
White	23 (85.2)	102 (69.9)	125 (72.3)
Other	0	3 (2.1)	3 (1.7)
Ethnicity, n (%)			
Hispanic or Latino	2 (7.4)	10 (6.8)	12 (6.9)
Not Hispanic or Latino	25 (92.6)	135 (92.5)	160 (92.5)
Not reported	0	1 (0.7)	1 (0.6)
Geographical region, n (%)			
United States	4 (14.8)	18 (12.3)	22 (12.7)
Japan	1 (3.7)	9 (6.2)	10 (5.8)
Europe	16 (59.3)	69 (47.3)	85 (49.1)
Rest of the world	6 (22.2)	50 (34.2)	56 (32.4)

Abbreviations: max, maximum; min, minimum; N, total number of subjects in the Efficacy Population (overall or in a given treatment group); n, number of subjects within a given criterion.

- **Numbers analysed**

Of the 175 subjects enrolled, 174 subjects were treated with rVIII-SingleChain and comprised the Safety Population, and 173 subjects comprised the Efficacy Population (146 subjects in the prophylaxis group and 27 subjects in the on-demand group). The PK Population comprised a total of 91 subjects (27 subjects in the Part 1 PK Population and 64 subjects in the Part 3 PK Population). The Surgical Population comprised 13 subjects (3 subjects in the on-demand group and 10 subjects in the prophylaxis group) who underwent a total of 16 surgeries. The PP Population comprised 156 subjects. A summary of subject disposition for the Efficacy population is presented (by treatment modality and total) in Table 9.

There were no deaths, and no subjects discontinued from the study due to AEs, lack of efficacy, loss to follow-up, protocol violations, or unknown reasons.

Table 9 Subject Disposition (Efficacy Population)

	On-demand (N=27)	Prophylaxis (N=146)	Total (N=173)
Efficacy population	27	146	173
Completed study	21 (77.8)	140 (95.9)	161 (93.1)
Discontinued from study	6 (22.2)	6 (4.1)	12 (6.9)
Reasons for discontinuation			
Withdrawal by subject	1 (3.7)	6 (4.1)	7 (4.0)
Other	4 (14.8)	0	4 (2.3)
Physician decision	1 (3.7)	0	1 (0.6)

Notes:
[1] Table presents number and percentage of subjects (n [%]).
[2] Percentages are based on the number of subjects in the Efficacy Population.
Source: Tables 14.1.2.2.

- **Outcomes and estimation**
  - Efficacy in treatment of bleeding

There were 616 spontaneous bleeding episodes requiring treatment in Study 1001 (subjects ≥ 12 to ≤ 65 years of age).

Table 10 Overall Investigator's Assessment of Haemostatic Efficacy (Efficacy Population)

Bleeding type assessment	On-demand (N=27)	Prophylaxis (N=146)	Overall (N=173)
Total			
Number of bleeding episodes	594	278	872
Number of treated bleeding episodes	590	258	848
Excellent (n [%])	421 (71.4)	182 (70.5)	603 (71.1)
Good (n [%])	124 (21.0)	56 (21.7)	180 (21.2)
Moderate (n [%])	32 (5.4)	20 (7.8)	52 (6.1)
Poor / no response	0	0	0
Missing	13 (2.2)	0	13 (1.5)
Treatment success (a)	545 (92.4)	238 (92.2)	783 (92.3)
Rate of treatment success	92.4	92.2	92.3
95% CI for rate	(87.8, 95.3)	(86.3, 95.8)	(88.9, 94.8)
Treatment success (b)	545 (94.5)	238 (92.2)	783 (93.8)
Rate of treatment success	94.5	92.2	93.8
95% CI for rate	(90.9, 96.7)	(86.3, 95.8)	(91.0, 95.7)
Treatment success (c)	558 (94.6)	238 (92.2)	796 (93.9)
Rate of treatment success	94.6	92.2	93.9
95% CI for rate	(91.0, 96.8)	(86.3, 95.8)	(91.1, 95.8)

Notes:
[1] Success is defined as a rating of excellent or good. [a] Primary analysis: missing counted as treatment failure; [b] Sensitivity analysis: all missing excluded. [c] Sensitivity analysis: missing counted as treatment success.
[2] 95% CI based on a generalized linear model to account for within-subject correlation.
[3] Table presents number and percentage of bleeding episodes [(n(%)].
[4] Percentages are based on the number of treated bleeding episodes.

Of the 848 treated bleeding episodes, 835 were assessed by the investigator for haemostatic efficacy, and 783 were assessed as “excellent” or “good” (ie, as treatment successes). In the primary analysis (ie, missing assessments counted as treatment failures), the rate of treatment success was 92.3%. When missing assessments were excluded, the rate of treatment success was 93.8% overall. The rate of treatment success was similar between treatment regimens (94.5% [on-demand] and 92.2% [prophylaxis]), and between the 2 age groups (95.7%[≥ 12 to < 18 years] and 93.7% [≥ 18 to ≤ 65 years]).

All treated bleeding episodes were considered as minor or moderate. In 93.5% of treated bleeding episodes, 1 or 2 injections of rVIII-SingleChain were sufficient to achieve hemostasis.

Table 11 Number of rVIII-SingleChain Injections Required to Achieve Hemostasis (Efficacy Population)

	On-demand (N=27)	Prophylaxis (N=146)	Overall (N=173)
Number of bleeding episodes	594	278	872
Number of bleeding treated episodes	590	258	848
Number of subjects with ≥ 1 bleeding episode	26	85	111
Number of subjects with ≥ 1 treated bleeding episode	26	83	109
Number of injections required to achieve hemostasis (n [%]))			
1 injection	488 (82.7)	198 (76.7)	686 (80.9)
2 injections	71 (12.0)	36 (14.0)	107 (12.6)
3 injections	19 (3.22)	10 (3.88)	29 (3.42)
> 3 injections	12 (2.03)	14 (5.43)	26 (3.07)

Notes:
[1] Percentages for bleeding episodes are based on the total number of bleeding episodes treated.
[2] *n=number of treated bleeding episodes.

Efficacy: Routine Prophylaxis to Prevent or Reduce the Frequency of Bleeding Episodes

The median observed AsBR and ABR was low in this study (0.00 and 1.14, respectively). The AsBR in subjects on the prophylaxis regimen was significantly lower ($p < 0.0001$) than in subjects on the on-demand regimen. Similar results were observed for the ABR, and for the AsBR and ABR in subjects ⩾ 18 to ⩽ 65 years. In subjects ⩾ 12 to < 18 years, the AsBR and ABR could not be compared between regimens, as all subjects in this age group were using the prophylaxis regimen.

The majority of subjects using a prophylaxis regimen were administered rVIII-SingleChain 2 or 3 times weekly at 20 to 50 IU/kg. The AsBR and ABR were similar between these 2 prophylaxis regimens. For any prophylaxis regimen, most subjects did not have a dose adjustment or only had 1 dose adjustment (87.7%).

Table 12: Annualized Spontaneous Bleeding Rate – rVIII-SingleChain Prophylaxis Compared with rVIII-SingleChain On-demand (Efficacy Population)

	On-demand (N=27)	Prophylaxis (N=146)
Spontaneous bleeding episodes		
n	27	146
Mean (SD)	24.84 (33.843)	2.10 (4.764)
Median	11.73	0.00
Q1, Q3	2.8, 36.5	0.0, 2.4
Min, Max	0.0, 151.0	0.0, 40.6
Number of bleeding episode per year (95% CI)	19.5 (17.8, 21.3)	1.6 (1.3, 1.8)
P-value	< 0.0001	
Prophylaxis / On-demand ratio (95% CI)	0.08 (0.07, 0.10)	
Total bleeding episodes		
n	27	146
Mean (SD)	31.14 (35.560)	3.11 (5.045)
Median	19.64	1.14
Q1, Q3	6.2, 46.5	0.0, 4.2
Min, Max	0.0, 163.3	0.0, 40.6
Number of bleeding episodes per year (95% CI)	24.9 (23.0, 27.0)	2.6 (2.3, 2.9)
P-value	< 0.0001	
Prophylaxis / On-demand ratio (95% CI)	0.10 (0.09, 0.12)	

SD, standard deviation.
Note:
[1] Number of bleeding episodes per year (95% CI), p-value and ratio are based on Poisson distribution.

A summary of the consumption of rVIII-SingleChain during routine prophylaxis is presented in Table 13. A mean (SD) prophylaxis dose of 374.5 (148.18) IU/kg was administered per month.

Table 13: Consumption of rVIII-SingleChain During Routine Prophylaxis (Efficacy Population)

	Prophylaxis (N=146)
Number of subjects on routine prophylaxis treatment [n (%)]	**146**
Total number of prophylaxis injections	**12343**
Prophylaxis dose [1] per subject per month (IU/kg)	
n	146
Mean (SD)	374.5 (148.18)
Median	356.9
Min, Max	31, 1522
Total dose [1] per subject per month (IU/kg)	
n	146
Mean (SD)	417.9 (194.11)
Median	388.5
Min, Max	173, 1732
Prophylaxis dose [1] per subject per year (IU/kg)	
n	146
Mean (SD)	4494.4 (1778.17)
Median	4282.9
Min, Max	372, 18263
Total dose [1] per subject per year (IU/kg)	
n	146
Mean (SD)	5015.0 (2329.33)
Median	4661.5
Min, Max	2078, 20789
Prophylaxis IU per month	
n	146
Mean (SD)	28065.5 (14648.99)
Median	26686.8
Min, Max	2792, 136969
Total IU per month	
n	146
Mean (SD)	31598 (19149.31)
Median	27706.4
Min, Max	8677, 155916

SD, standard deviation.

Notes:

[1] Prophylaxis injections are those recorded as administered for 'routine / prophylaxis'. Total injections also include those recorded as administered for 'bleeding event', 'surgery', 'post Surgery', 'prevention prior to activity', and 'additional treatment'.

A summary of dose assignment and dose adjustment for subjects receiving rVIII-SingleChain prophylaxis is presented in Table 14.

Table 14: Summary of Dose Assignment and Dose Adjustment in Subjects on Prophylaxis Regimens (Efficacy Population)

	Every 2nd day (N=9)	3 times per Week (N=79)	2 times per Week (N=47)	Other regimen (N=11)
Assigned dose (IU/kg)				
n	9	79	47	11
Mean (SD)	31.0 (7.55)	31.9 (8.45)	35.5 (8.52)	33.3 (10.79)
Median	30.0	30.0	35.0	30.0
Min, Max	20, 42	12, 50	17, 50	17, 46
Number of dose adjustments per subject [n (%)]				
0	7 (77.8)	52 (65.8)	34 (72.3)	7 (63.6)
1	2 (22.2)	15 (19.0)	9 (19.1)	2 (18.2)
2	0	8 (10.1)	3 (6.4)	1 (9.1)
> 2	0	4 (5.1)	1 (2.1)	1 (9.1)
Number of subjects assigned a dose < 20 IU/kg **[n (%)]**	0	2 (2.5)	1 (2.1)	2 (18.2)
Number of subjects assigned a dose > 50 IU/kg **[n (%)]**	0	1 (1.3)	0	0
Reason for dose adjustment [n (%)]				
Bleeding	0	5 (6.3)	0	0
Physician decision	2 (22.2)	14 (17.7)	9 (19.1)	2 (27.3)
Other	0	15 (19.0)	3 (6.4)	2 (18.2)

SD, standard deviation.
Notes:
[1] Where indicated, table presents number and percentage of subjects [(n(%)].
[2] Percentages are based on the number of subjects in the respective group.
[3] Number of subjects assigned a dose < 20 IU/kg or > 50 IU/kg includes initial dose assigned and dose adjustments assigned.
Source: Table 14.1.10.

The majority of subjects received prophylaxis rVIII-SingleChain administered at 20-50 IU/kg 2 or 3 times per week.

Efficacy: Perioperative Prophylaxis (Surgical Prophylaxis)

There were 16 surgeries during the study, all of which were non-emergency surgeries. The treatment success rate of rVIII-SingleChain was 100%, based on the investigator's overall clinical assessment of haemostatic efficacy. The mean volume of blood loss during the surgical substudy was lower than predicted levels, while the volume of transfused packed red blood cells was consistent with predicted levels. Hemoglobin levels were maintained close to baseline levels, with a slight reduction in the post-operative period.

Doses of rVIII-SingleChain administered during the surgical substudy were in the range expected to achieve efficacy based on the WFH guidelines, both pre- and intra-operatively (pre-surgery 68.3 [22.16] IU/kg, intra-operative 37.0 [11.81] IU/kg) and during the 14 days of the post-surgical period (704.0 [391.74] IU/kg)(mean [SD] values). rVIII-SingleChain consumption in surgery was within the expectations for the types of procedures performed.

The volume (mean [SD]) of blood loss during the surgical substudy (73.3 [107.18] mL) was lower than predicted volume (259.3 [369.42] mL).

Table 15 Efficacy of rVIII-SingleChain in Perioperative Prophylaxis (Surgical Population, Study 1001)

Procedure	Efficacy evaluation	Factor consumption (IU/kg) pre- and intra-operatively
Extraction of wisdom teeth	Excellent	51.09
Abdominal hernia repair	Excellent	47.89
Elbow replacement	Excellent	108.58
Ankle arthroplasty	Excellent	76.83
Knee replacement (5)	Excellent (4), Good (1)	92.49 100.9 67.26 105.79 86.09
Cholecystectomy and Lengthening of achilles tendon combined with: Straightening of right toes	Excellent Excellent	105.95
Circumcision (3)	Excellent (3)	99.04 92.74 81.5
ORIF right ankle	Excellent	89.36
Hardware removal, right ankle	Excellent	40.45

Abbreviations: IU, International Units; ORIF, open reduction internal fixation; rVIII-SingleChain, recombinant single-chain factor VIII.

Source: Study 1001 CSR, Table 14.2.5.4, Listing 16.2.6.2, and Appendix 16.3, SDTM.YE

- **Ancillary analyses**

n/a

- **Summary of main efficacy results**

The following table summarises the efficacy results from study 1001 supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 16: Summary of Efficacy for study 1001

Title: A Phase I/III Open-label, Multicenter, Crossover Safety, Efficacy and Pharmacokinetic Study of Recombinant Coagulation Factor VIII (rFVIII) Compared to Recombinant human antihaemophilic factor VIII (rFVIII; INN: octocog alfa) in Subjects with Haemophilia A, and a Repeat PK, Safety and Efficacy Study		
Study identifier	CSL627_ 1001	
Design	This is an open-label, non-randomized, efficacy, safety and pharmacokinetic (PK) study comparing octocog alfa and rVIII-SingleChain. The study consists of three parts, a PK period (Part 1), a continuation of dosing safety and efficacy period (Part 2) and a safety, efficacy, and repeat PK period (Part 3) and also includes a surgical sub-study for subjects enrolled in Parts 2 and 3.	
	Duration of main phase:	2 years 9 months

	Duration of Run-in phase:		NA
	Duration of Extension phase:		NA
Hypothesis	The study is being conducted to support the approval of rVIII-SingleChain, a new and novel recombinant FVIII for the following indications: 1. Prevention and treatment of hemorrhagic episodes in patients with haemophilia A. 2. Surgical prophylaxis of bleeding episodes in patients with haemophilia A. 3. Routine prophylaxis of bleeding episodes in patients with haemophilia A.		
Treatments groups	rVIII-SingleChain Prophylaxis		The rVIII-SingleChain Prophylaxis group consisted of all subjects in the Efficacy population who received at least 1 dose rVIII-SingleChain as part of routine prophylaxis treatment during Parts 2 or 3 of the study. There were 146 subjects in the rVIII-SingleChain Prophylaxis group.
	rVIII-SingleChain On-Demand		The rVIII-SingleChain On-demand group consisted of all subjects in the Efficacy population who received at least 1 dose rVIII-SingleChain as part of on-demand treatment during Parts 2 or 3 of the study. There were 27 subjects in the rVIII-SingleChain On-demand group.
	rVIII-SingleChain		The Efficacy population consisted of all subjects who received at least 1 dose of rVIII-SingleChain as part of either routine prophylaxis treatment or on-demand treatment during Parts 2 or 3 of the study. There were 173 subjects in the Efficacy population.
Endpoints and definitions	Primary	Treatment Success	The investigator rated the efficacy of the treatment based on a 4-point rating scale "excellent, good, moderate or poor/no response". Efficacy ratings of "excellent" or "good" were considered treatment success for this end point; the percentage of bleeding events with a rating of excellent or good and the 95% confidence interval are presented. The denominator includes all treated bleeding events. The 95% confidence interval is based on a generalized linear model to account for within-subject correlation.
	Primary	Inhibitor formation to FVIII	Number of subjects who develop inhibitors to FVIII
	Primary	Annualized Spontaneous Bleeding Rate (AsBR)	The annualized spontaneous bleeding rate (AsBR) was derived for each subject as follows: 365.25*(number of spontaneous bleeding episodes requiring treatment) / (observed treatment period of interest)
	Primary	Treatment Success During the Peri-operative Surgical Sub-study	Subjects received rVIII-SingleChain before and during surgery based on the type of surgery and the clinical status of the subject. The investigator rated the efficacy of the treatment based on a 4-point surgical treatment rating scale of "excellent, good, moderate or poor/no response". Efficacy ratings of "excellent" or "good" were considered treatment success for this end point. The rate of success, defined as the percentage of surgeries with a rating of excellent or good for haemostatic efficacy on the surgical treatment scale is presented for the Surgical Population, based on the total number of surgeries (N=16) as denominator
	Secondary	Annualized Bleeding Rate (ABR) for Total Bleeds and Traumatic Bleeds	The annualized bleeding rate was derived for each subject as follows: 365.25*(number of bleeding episodes requiring treatment) / (observed treatment period of interest)

	Secondary	Proportion of Bleeding Episodes (BE) Requiring 1, 2, 3 or > 3 Infusions of rVIII-SingleChain to Achieve Hemostasis	Percentage of bleeding episodes requiring 1, 2, 3 or > 3 infusions of rVIII-SingleChain to achieve hemostasis. The denominator includes all treated bleeding episodes		
Database lock	23-Jan-15				
Results and Analysis					
Analysis description	Primary Analysis				
Analysis population and time point description	Efficacy				
Descriptive statistics and estimate variability	Treatment group	rVIII-SingleChain On-Demand	rVIII-SingleChain Prophylaxis	rVIII-SingleChain	rVIII-SingleChain Surgical
	Number of subjects	27	146	173	13
	Number of Treated Bleeding Events	590	258	848	NA
	Treatment Success [% bleeding events successfully treated (95% CI)]	92.4 (87.8 to 95.3)	92.2 (86.3 to 95.8)	92.3 (88.9 to 94.8)	NA
	Inhibitor formation to FVIII (subjects)	0	0	0	NA
	AsBR [Number of spontaneous bleeds per year, Median (Inter-Quartile Range)]	11.73 (2.8 to 36.5)	0.00 (0.0 to 2.4)	NA	NA
	Surgical Sub-study Treatment Success (%)	NA	NA	NA	100
	ABR for Total Bleeds [Number of bleeds per year] Median (Inter-Quartile Range)	19.64 (6.2 to 46.5)	1.14 (0.0 to 4.2)	NA	NA

	ABR for Traumatic Bleeds [Number of bleeds per year, Median (Inter-Quartile Range)]	3.12 (0.0 to 8.4)	0.00 (0.0 to 0.9)	NA	NA
	Bleeding Episodes (BE) Requiring 1 infusion (Percentage of bleeding episodes)	82.7	76.7	80.9	NA
	BE Requiring 2 infusions (Percentage of bleeding episodes)	12.0	14.0	12.6	NA
	BE Requiring 3 infusions (Percentage of bleeding episodes)	3.22	3.88	3.42	NA
	BE Requiring >3 infusions (Percentage of bleeding episodes)	2.03	5.43	3.07	NA

Study 3002 (Subjects 0 to < 12 Years)

Study 3002 was a multicenter, open-label, phase III study to assess the efficacy, safety, and PK of rVIII-SingleChain in subjects 0 to < 12 years of age with severe haemophilia A. The study consisted of a PK evaluation period (single-dose PK of 50 IU/kg rVIII-SingleChain) and a treatment period (on-demand or prophylaxis regimen with rVIII-SingleChain).

Methods

- **Study participants**

Key Inclusion Criteria

Subjects who met all of the following inclusion criteria were eligible for enrolment into the study:

- Diagnosis of severe haemophilia A defined as < 1% FVIII concentration (FVIII:C) documented in medical records
- Males < 12 years of age
- Subjects who had received > 50 EDs with a FVIII product
- Written informed parental or guardian consent and assent of minors for study participation obtained before undergoing any study specific procedures
- Prior PK data (at least IR and t1/2) from previous FVIII exposure for subjects participating in the PK assessment

Key Exclusion Criteria

Subjects who met any of the following exclusion criteria were not eligible for enrolment into the study:

- Any history of, or current, FVIII inhibitors
- Any first order family (ie, siblings) history of FVIII inhibitors
- Administration of any cryoprecipitate, whole blood, or plasma within 30 days prior to administration of rVIII-SingleChain
- Known hypersensitivity (allergic reaction or anaphylaxis) to any FVIII product or hamster protein
- Any known congenital or acquired coagulation disorder other than congenital FVIII deficiency
- Platelet count < 100,000/µL at Screening
- Human immunodeficiency virus (HIV) positive subjects with a CD4 count < 200/mm3 at Screening
- Subject currently receiving intravenous (IV) immunomodulating agents such as immunoglobulin or chronic systemic corticosteroid treatment
- Subject with serum aspartate aminotransferase or serum alanine aminotransferase values > 5 times (x) the upper limit of normal at Screening
- Subjects with serum creatinine values > 2 x the upper limit of normal at Screening
- Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months before Day 1
- Known or suspected hypersensitivity to rVIII-SingleChain or to any excipients of rVIII-SingleChain

- **Treatments**

In the treatment period, subjects were assigned to an on-demand or prophylaxis regimen with rVIII-SingleChain at the investigator's discretion. In the event of a bleeding episode, subjects in both regimens were treated with a rVIII-SingleChain dose prescribed by the investigator. The desired FVIII level for the treatment of a bleeding episode was based on the recommendations of the WFH (WFH, 2012).

For the prophylaxis regimen, subjects were to receive rVIII-SingleChain at a dose of 15 to 50 IU/kg every second day or 2 to 3 times weekly, or at a dose and frequency determined by the investigator based on historical FVIII dosing and available PK data. Subjects were not allowed to switch from on-demand to prophylaxis regimen or vice versa, but dose adjustments were permitted at the discretion of the investigator. In addition to their on-demand or prophylaxis regimen, subjects could receive preventative doses (eg, doses given prior to an activity or minor procedure to prevent or reduce potential bleeding episodes) or additional doses (eg, doses taken beyond the need to control hemostasis). These doses contributed to an ED and consumption of FVIII, but did not contribute to the efficacy evaluation of the treatment of a bleeding episode. Subjects were to be treated for at least 50 EDs until there was a total of 25 subjects 0 to < 6 years and 25 subjects ≥ 6 to < 12 years achieving at least 50 EDs in the respective age group, thereafter, subjects could roll over into Extension study 3001.

- **Objectives**

The primary objective of the study was to evaluate the efficacy of rVIII-SingleChain in the treatment of major and minor bleeding episodes based on the investigator's 4-point assessment scale.

- **Outcomes / endpoints**

The primary efficacy endpoint was:

- Control and prevention of bleeding episodes: rate of treatment success for bleeding episodes defined as a rating of "excellent" or "good" on the investigator's overall clinical assessment of haemostatic efficacy 4-point scale

To estimate the rate of treatment success, the numerator included the number of bleeding episodes treated with rVIII-SingleChain and rated as "excellent" or "good", and the denominator included all treated bleeding episodes. In the primary analysis, treated bleeding episodes with missing investigator ratings were counted as treatment failures. In the sensitivity analyses, bleeding episodes with missing investigator ratings were either excluded from the calculation, or counted as treatment success.

The secondary efficacy endpoints were:

- Control and prevention of bleeding episodes: number of injections of rVIII-SingleChain required to achieve hemostasis, and consumption of rVIII-SingleChain
- Routine prophylaxis: ABR during on-demand and during prophylaxis treatment, and consumption of rVIII-SingleChain

Other secondary endpoints were:

- PK parameters for rVIII-SingleChain

The secondary safety endpoints:

- Occurrence of inhibitor formation to rVIII-SingleChain evaluated from the time of first rVIII-SingleChain dose through the End-of-study visit
- Safety measures including AEs, SAEs, local tolerability, laboratory safety parameters, measurement of non-inhibitory ADAs and antibodies against CHO host cell proteins, physical examination, and vital signs (blood pressure, heart rate, temperature, and respiratory rate)

- **Sample size**

The determination of sample size was based on the EMA guideline for recombinant and human plasma-derived Factor VIII products in children < 12 years of age [EMA, 2011]. This guideline requires a minimum of 25 subjects ≥ 6 years of age to < 12 years of age and 25 subjects 0 to < 6 years of age suffering from severe haemophilia A. A total of approximately 75 subjects were planned to be enrolled in this study to ensure that at least 25 subjects in each age group receive 50 EDs of rVIII-SingleChain.

No formal statistical comparisons were planned in this study. Rather, the primary aim of analyses was to provide descriptive summaries, and in some cases point and interval estimates, of key variables or parameters.

- **Randomisation**

Not applicable.

- **Blinding (masking)**

Not applicable.

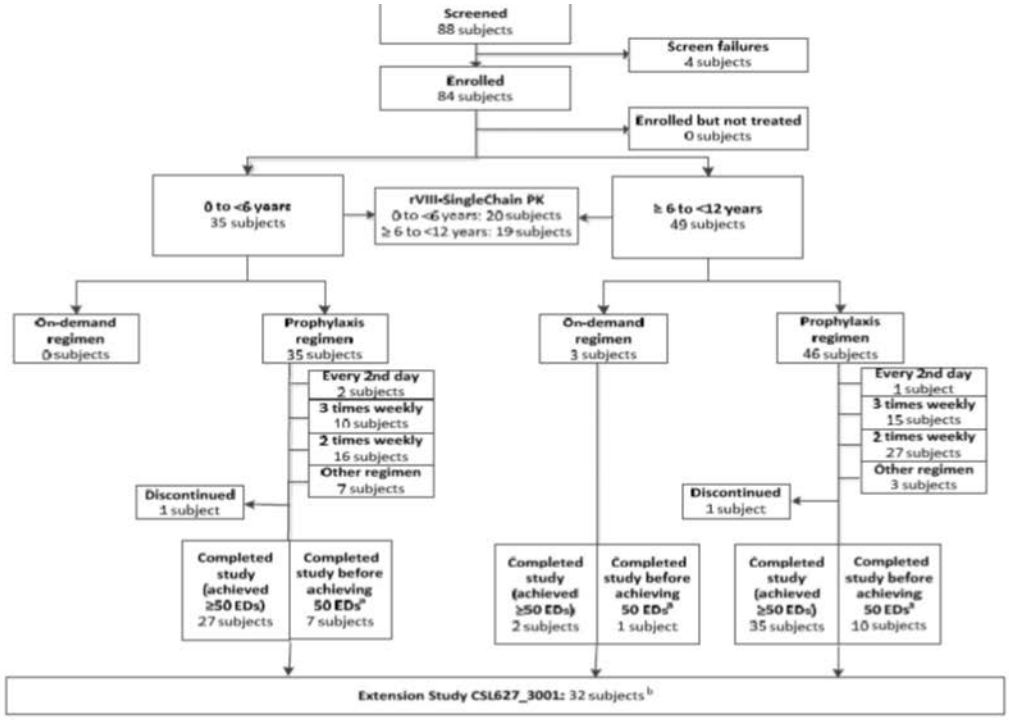
- **Statistical methods**

In study CLS627-3002 no statistical testing was planned. Instead point estimates and 2-sided 95%-CIs were provided for selected parameters. Variables were summarized in terms of statistical characteristics (continuous variables: mean, standard deviation, median, minimum and maximum; categorical variables: absolute and relative frequencies) overall as well as for pre-defined subgroups.

Results

- **Participant flow**

Figure 3.4.3 Disposition of Subjects (All Subjects, Study 3002)



Abbreviations: EDs, exposure days; PK, pharmacokinetic(s); rVIII-SingleChain, recombinant single-chain factor VIII

[a] Subjects completed the study since the primary objective, ie, 25 subjects achieving at least 50 EDs in the respective age cohort, was achieved, and subjects could roll over to Extension Study 3001 where the study was open for enrollment.

- **Recruitment**

A total of 88 subjects were screened for this study at 37 study sites in 19 countries. Eighty-four of the screened subjects were eligible and enrolled into the study (35 subjects 0 to < 6 years; 49 subjects ≥ 6 to < 12 years), and all 84 subjects were exposed to treatment with rVIII-SingleChain. Eighty-one subjects were assigned to a prophylaxis regimen, the remaining 3 subjects were assigned to on-demand regimen (all in the ≥ 6 to < 12 years age group).

Overall, 65 subjects completed the study, 19 subjects were discontinued from the study (1 subject due to an AE, 1 subject based on physician decision, and 17 subjects due to study termination by the sponsor [Note: "study termination by the sponsor" signifies the planned closure of the study in an age group once the required number of 25 subjects with 50 EDs had been reached in that age group. Subjects still on study at this time were rolled over into Extension Study 3001, irrespective of their number of EDs). One

subject was discontinued by the physician due to a series of complex social circumstances; this case was reviewed by the IDMC.

There was no difference in the frequency or reasons of discontinuation between the Safety and Efficacy Populations, between the 2 age groups, or between the on-demand and prophylaxis regimens.

Table 17: Subject Disposition by Age Group (Safety Population)

	Number (%) of subjects		
	< 6 years (N = 35)	≥ 6 to < 12 years (N = 49)	Total (N = 84)
Completed study	27 (77.1)	38 (77.6)	65 (77.4)
Discontinued from study	8 (22.9)	11 (22.4)	19 (22.6)
Reasons for discontinuation			
AEs	0	1 (2.0)	1 (1.2)
Physician decision	1 (2.9)	0	1 (1.2)
Study terminated by sponsor [a]	7 (20.0)	10 (20.4)	17 (20.2)

Abbreviations: AE, adverse event; EDs, exposure days; N, total number of subjects (overall or within a given age group).
[a] Planned closure of the age group once the required number of 25 subjects with 50 EDs had been reached in that age group. Subjects still on study at this time were rolled over into Extension Study 3001, irrespective of their number of EDs achieved in Study 3002.

- **Conduct of the study**

Four protocol amendments (2 global substantial amendments, 1 non-substantial amendment [administrative change] and 1 country specific amendment for France) were issued after finalization of Version 1.0 of the study protocol on 05 October 2012. No subjects were enrolled under Version 1.0 of the study protocol and that enrollment started under Protocol Amendment 1.

The main changes to the study conduct as a result of the global substantial protocol amendments were as follows:

Protocol Amendment 1 was issued on 21 May 2013:

Extend the duration of subject study participation to allow at least 50 EDs; Increase cohort size of subjects screened for participation; Distinguish lab assessments performed by local or central laboratories; Reiterate the recording of actual dosing rather than nominal dosing; Add assessment for CHO antibodies; Update the definitions of overdose, treatment compliance, retention of samples, and haemophilia social history and activity level; Clarify End-of-study procedures; Define preventative dosing and additional dosing; Collect additional subject information including blood group and gene defect of haemophilia A; Clarify serum chemistry and hematology laboratory parameters to be collected; Clarify statistical analyses and methods;Update IDMC responsibilities

Protocol Amendment 2 was issued on 28 March 2014:

Incorporate the non-substantial amendment change for subjects not returning unused medication at every visit; Incorporate a change in the PK collection time points as recommended by FDA; Update IDMC data review information

The following changes were made to the statistical analyses between the SAP and the analyses performed:

- The Efficacy Population had been defined as all subjects (in the Safety Population) who received at least 1 dose of Afstyla as part of either routine prophylaxis or on-demand regimen. The Efficacy Population excluded 1 subject who had a positive test for FVIII inhibitors at screening.

- Prophylaxis IU and total IU per subject per month and per year were not calculated, as the body weight-adjusted data (IU/kg) were considered to provide sufficient, and more meaningful, information about monthly and annual exposure.
- The number of spontaneous bleeding episodes in last 12 months, number of trauma-induced bleeding episodes in last 12 months, and number of bleeding episodes of unknown causality in last 12 months were only listed by subject, not summarized.
- Prophylaxis compliance was not summarized as a continuous variable, but only as a categorical variable.
- Annualized bleed rate was additionally summarized for the following subgroups:
  - Subjects whose initial dose assignment was 15 to 50 IU/kg
  - Subjects whose initial dose assignment was a once-weekly prophylaxis regimen
  - Subjects with a positive test for non-inhibitory ADAs anytime during the study
  - Subjects with zero dose adjustments; subjects with at least one dose adjustment
  - Subgroups based on reason for at least one dose adjustment: physician decision, lack of efficacy, or other reasons
  - Subjects with zero dose adjustments and ≥ 2 spontaneous bleeding episodes within a 14-day period ; subjects with zero dose adjustments who did not have≥ 2 spontaneous bleeding episodes within a 14-day period
  - Subgroups based on previous treatment modality: on-demand or prophylaxis
- The number of bleeding episodes over time was summarized for the time intervals of Day 1 to 90, Day 91 to 180, Day 181 to 270, and Day 271 to 360, instead of Day 1 to 90, Day 91 to 120, Day 121 to 150, etc. Day 631 to 720, Day > 720. Also, the number of bleeding episodes over time was only summarized for the Efficacy Population by modality (not overall and not by age group)
- The listing for time between the last injection and the next bleeding episode did not include the information on treatment modality
- There were no subgroup analyses for BMI ≥ 30 kg/m2, Japan, and Black subjects as there were no subjects in these subgroups.
- Clinically significant vital signs were reported based on criteria predefined in the SAP instead of based on investigator's assessment of clinical significance, because investigator's assessment of clinical significance was not collected.

- **Baseline data**

The subject population was all male and predominantly White (73.5% in the Efficacy Population). Subjects had a mean age of 6.6 years (minimum, maximum: 1, 11 years). Differences between subjects 0 to < 6 years and subjects ≥ 6 to < 12 years were consistent with the ontogeny of the subject population.

Subjects had a mean weight of 27.6 kg.

The demographic and baseline characteristics of the Efficacy, Safety, and PK Populations were generally consistent, except for a higher proportion of White subjects in the PK Population compared to the Efficacy and Safety Populations.

Table 18 Demographic Characteristics of Pediatric Subjects by Age Subgroup (Safety Population, Study 3002)

Characteristics	0 to < 6 years (N = 35)	≥ 6 to < 12 years (N = 49)	Overall (N = 84)
Age, years			
Mean (SD)	3.5 (1.34)	8.8 (1.77)	6.6 (3.11)
Median	4.0	9.0	7.0
Min, max	1, 5	6, 11	1, 11
Weight, kg			
Mean (SD)	16.62 (3.566)	35.45 (12.361)	27.60 (13.447)
Median	16.00	32.00	25.00
Min, max	10.0, 26.2	18.7, 87.5	10.0, 87.5
Race, n (%)			
Asian	9 (25.7)	13 (26.5)	22 (26.2)
White	25 (71.4)	36 (73.5)	61 (72.6)
Other	1 (2.9)	0	1 (1.2)
Ethnicity, n (%)			
Hispanic or Latino	1 (2.9)	1 (2.0)	2 (2.4)
Not Hispanic or Latino	33 (94.3)	48 (98.0)	81 (96.4)
Not reported	1 (2.9)	0	1 (1.2)
Geographical region, n (%)			
United States	2 (5.7)	2 (4.1)	4 (4.8)
Europe	21 (60.0)	28 (57.1)	49 (58.3)
Rest of the world	12 (34.3)	19 (38.8)	31 (36.9)

Abbreviations: max, maximum; min, minimum; N, total number of subjects in the Safety Population (overall or in a given age group); n, number of subjects within a given criterion.

- **Numbers analysed**

A total of 84 subjects (35 subjects 0 to < 6 years, and 49 subjects ≥ 6 to < 12 years) were treated with rVIII-SingleChain and comprised the Safety Population. The Efficacy Population comprised 83 subjects (80 subjects assigned to a prophylaxis regimen, and 3 subjects assigned to an on-demand regimen). The PK Population comprised 39 subjects (20 subjects 0 to < 6 years, 19 subjects ≥ 6 to < 12 years).

There were no deaths. Two subjects were withdrawn from the study (1 subject due to an unrelated non-serious TEAE of hip arthralgia, 1 subject based on physician decision).

Table 19: Subject Populations (Enrolled Population, study 3002)

	Number of subjects		
	0 to < 6 years	≥ 6 to < 12 years	Total
Enrolled	35	49	84
Safety Population	35	49	84
Efficacy Population	35	48	83
PP Population	31	44	75
PK Population	20	19	39

Abbreviations: PP, per protocol; PK, pharmacokinetic.

- **Outcomes and estimation**

Overall, 80 of the 83 subjects in the Efficacy Population were assigned to a prophylaxis regimen with rVIII-SingleChain. The remaining 3 subjects were assigned to an on-demand regimen with rVIII-SingleChain. These 3 subjects were in the ≥ 6 to < 12 year group and had also been on an on-demand regimen before the study. 21 of the 80 subjects assigned to a prophylaxis regimen in this study had been on an on-demand regimen before the study.

The most frequent prophylaxis regimens in both age groups were the 2-times-weekly (43 subjects) and 3-times-weekly (24 subjects) regimens at the initial assignment. At the end of the study, 38 subjects were assigned to a 2-times-weekly regimen and 32 subjects to a 3-times-weekly regimen.

Across all prophylaxis regimens, 74 of the 80 subjects were assigned to an initial dose between 15 and 50 IU/kg, with only 1 subject assigned a dose less than 20 IU/kg. The remaining prophylaxis subjects assigned to an initial dose outside of the 15 to 50 IU/kg range used rVIII-SingleChain doses no higher than 57 IU/kg. No subjects used doses < 15 IU/kg.

Table 20: Summary of Dose Assignment and Dose Adjustments (Efficacy Population, study 3002)

	On-demand regimen (N = 3)	Assigned Prophylaxis Regimen				Total (N = 83)
		Every 2nd day (N = 3)	3 times per Week (N = 24)	2 times per Week (N = 43)	Other regimen (N = 10)	
Initial assigned dose, IU/kg						
N	3	3	24	43	10	83
Mean (SD)	26.0 (2.65)	39.0 (11.53)	33.8 (9.64)	34.7 (10.12)	43.0 (12.73)	35.3 (10.56)
Median	25.0	40.0	32.0	35.0	50.0	34.0
Min, Max	24, 29	27, 50	19, 50	20, 57	23, 55	19, 57
Number of subjects assigned dose < 15 IU/kg, n (%)	0	0	0	0	0	0
Number of subjects assigned dose > 50 IU/kg, n (%)	0	0	0	4 (9.3)	3 (30.0)	7 (8.4)
Final assigned dose, IU/kg						
N	3	3	32	38	7	83
Mean (SD)	25.0 (4.00)	44.3 (5.13)	34.9 (10.02)	36.2 (10.37)	43.1 (13.75)	36.2 (10.64)
Median	25.0	43.0	32.0	35.5	50.0	35.0
Min, Max	21, 29	40, 50	20, 50	20, 57	23, 55	20, 57
Number of dose adjustments per subject, n (%)						
0	2 (66.7)	2 (66.7)	14 (58.3)	26 (60.5)	7 (70.0)	51 (61.4)
1	1 (33.3)	1 (33.3)	7 (29.2)	10 (23.3)	2 (20.0)	21 (25.3)
2	0	0	3 (12.5)	2 (4.7)	0	5 (6.0)
> 2	0	0	0	5 (11.6)	1 (10.0)	6 (7.2)
Reason for dose adjustment, n (%)[a]						
Physician decision	1 (33.3)	1 (33.3)	8 (33.3)	13 (30.2)	3 (30.0)	26 (31.3)
Lack of efficacy	0	0	0	1 (2.3)	0	1 (1.2)
Other	0	0	4 (16.7)	4 (9.3)	0	8 (9.6)

Abbreviations: IU, international units; max, maximum; min, minimum; N, total number of subjects (overall or within a given regimen); n, number of subjects within a given criterion.

[a] For subjects who had > 1 dose adjustment for different reasons, the reason for each dose adjustment was included.

Notes:

[1] Where indicated, table presents number and percentage of subjects (n [%]). Percentages are based on the number of subjects in the respective group.

[2] Number of subjects assigned a dose < 15 IU/kg or > 50 IU/kg includes initial dose assigned and dose adjustments.

51 of the 83 subjects (61.4%) in the Efficacy Population required no adjustment of the rVIII SingleChain dose or regimen. A small number of subjects shifted from the 2-times-weekly (N = 6), every second day (N = 1) or once-weekly (N = 1) regimens to a 3-times-weekly regimen.

However, the percentage of prophylaxis subjects requiring ≥ 1 dose adjustment was similar between the 2-times-weekly regimen (39.5%) and the 3-times-weekly regimen (41.7%). The main reason for dose adjustments was “physician decision” in all treatment regimens.

A higher proportion of subjects in the ≥ 6 to < 12 year group had at least 1 dose adjustment (21 of 48 subjects [43.8%]) than in the 0 to < 6 year group (11 of 35 subjects [31.4%]), and 5 of the 6 subjects with more than 2 dose adjustments were in the ≥ 6 to < 12 year age group.

Overall, 92.5% of prophylaxis subjects were compliant with their prophylaxis regimen, and 90.4% of subjects were compliant with their treatment dose.

Efficacy: Control and Prevention of Bleeding Episodes

The characteristics of the bleeding episodes treated in the study are summarized by treatment regimen and additionally by age group in Table 21.

The primary endpoint for assessment of rVIII-SingleChain for treatment of bleeding episodes was treatment success, defined as a rating of “excellent” or “good” on the investigator’s overall clinical assessment of haemostatic efficacy 4-point scale.

Table 21: Treatment Success - Overall Investigator's Assessment of Haemostatic Efficacy (Efficacy Population, study 3002)

Bleeding type assessment	On-demand regimen (N = 3)	Prophylaxis regimen (N = 80)	Overall (N = 83)
Number of bleeding episodes	133	256	389
Number of treated bleeding episodes	132	215	347
Excellent, n (%)	132 (100.0)	164 (76.3)	296 (85.3)
Good, n (%)	0	38 (17.7)	38 (11.0)
Moderate, n (%)	0	12 (5.6)	12 (3.5)
Poor / no response	0	1 (0.5)	1 (0.3)
Treatment success (a)	132	202	334
Rate of treatment success	100.0	94.0	96.3
95% CI for rate	N/A	[87.8, 97.1]	[91.3, 98.4]
Treatment success (b)	132	202	334
Rate of treatment success	100.0	94.0	96.3
95% CI for rate	N/A	[87.8, 97.1]	[91.3, 98.4]
Treatment success (c)	132	202	334
Rate of treatment success	100.0	94.0	96.3
95% CI for rate	N/A	[87.8, 97.1]	[91.3, 98.4]

Abbreviations: CI, confidence interval; N, total number of subjects (overall or within a given regimen); n, number of subjects within a given criterion; N/A, not applicable

Notes:

[1] Treatment success is defined as a rating of excellent or good. (a) Primary analysis: missing counted as treatment failure; (b) Sensitivity analysis: all missing excluded. (c) Sensitivity analysis: missing counted as treatment success.

[2] 95% CI based on a generalized linear model to account for within-subject correlation.

[3] Table presents number and percentage of bleeding episodes [(n(%)].

[4] Percentages are based on the number of treated bleeding episodes.

The investigator assessment of haemostatic efficacy was "excellent" for 296 treated bleeding episodes, "good" for 38 bleeding episodes, "moderate" for 12 bleeding episodes, and "poor/no response" for 1 bleeding episode. Thus, the rate of treatment success was 96.3% (ie, 334 of 347 episodes), with a 95% CI of 91.3% to 98.4%.

The rate of treatment success was similar between the 2 age groups (0 to < 6 years: 94.0%; ≥ 6 to < 12 years: 96.6%).

The secondary endpoint for assessment of treatment of bleeding episodes was the number of injections of rVIII-SingleChain required to achieve hemostasis (1, 2, 3, or > 3 injections).

Table 22: Number of rVIII-SingleChain Injections Required to Achieve Hemostasis (Efficacy Population, study 3002)

	On-demand regimen (N = 3)	Prophylaxis regimen (N = 80)	Overall (N = 83)
Number of bleeding episodes	133	256	389
Number of treated bleeding episodes	132	215	347
Number of subjects with ≥ 1 bleeding episode	3	63	66
Number of subjects with ≥ 1 treated bleeding episode	3	59	62
Number of injections required to achieve hemostasis (% of treated bleeding episodes)			
1 injection	131 (99.2)	167 (77.7)	298 (85.9)
2 injections	0	34 (15.8)	34 (9.8)
3 injections	1 (0.8)	7 (3.3)	8 (2.3)
> 3 injections	0	7 (3.3)	7 (2.0)
Total IU/kg per bleeding episode			
Mean (SD)	27.8 (7.70)	47.2 (36.88)	39.8 (30.86)
Median (min, max)	25.9 (21, 78)	37.0 (16, 282)	27.6 (16, 282)
Total IU/kg per injection per bleeding episode			
Mean (SD)	27.4 (6.33)	34.1 (11.41)	31.5 (10.30)
Median (min, max)	25.9 (21, 57)	30.0 (16, 76)	27.3 (16, 76)

Abbreviations: IU, international units; max, maximum; min, minimum; N, total number of subjects (overall or within a given regimen).

Overall, 1 or 2 injections of rVIII-SingleChain were sufficient to achieve hemostasis in 332 of the 347 treated bleeding episodes (95.7%). The overall median dose was 27.6 IU/kg per bleeding episode and 27.3 IU/kg per injection per bleeding episode. Seven bleeding episodes (2.0%) in 4 subjects required > 3 rVIII-SingleChain injections to achieve hemostasis.

The proportion of bleeding episodes controlled with 1 or 2 rVIII-SingleChain injection was similar between the 2 age groups (0 to < 6 years: 94.0%; ≥ 6 to < 12 years: 96.0% of treated bleeding episodes).

For 17 bleeding episodes in 12 of the 83 subjects in the Efficacy Population (all in the prophylaxis group), a total of 50 additional rVIII-SingleChain doses were administered (ie, doses beyond the need to control hemostasis to prevent rebleeding and promote hematoma resorption), with a median of 2 additional doses per bleeding episode. Additional doses for bleeding episodes were more frequently administered in subjects ≥ 6 to < 12 years (9 subjects with a total of 14 bleeding episodes and a total of 45 additional doses) than in subjects 0 to < 6 years (3 subjects with a total of 3 bleeding episodes and a total of 5 additional doses.

Efficacy: Routine Prophylaxis to Prevent or Reduce the Frequency of Bleeding Episodes

In the 80 subjects on prophylaxis, the median observed ABR was 3.69 bleeding episodes per year for total bleeding episodes, and 0.00 for spontaneous bleeding episodes.

Table 22: Annualized Bleeding Rate by rVIII-SingleChain Regimen (Efficacy Population, study 3002)

	On-demand regimen (N = 3)	Prophylaxis regimen (N = 80)
Total bleeding episodes		
Mean (SD)	66.77 (27.702)	5.22 (5.557)
Median	78.56	3.69
Q1, Q3	35.12, 86.62	0.00, 7.20
Min, Max	35.1, 86.6	0.0, 23.7
Number of bleeding episodes per year [95% CI]	71.5 [60.3, 84.8]	5.5 [4.8, 6.3]
Spontaneous bleeding episodes		
Mean (SD)	24.83 (22.191)	1.70 (2.972)
Median	31.76	0.00
Q1, Q3	0.00, 42.73	0.00, 2.20
Min, Max	0.0, 42.7	0.0, 14.0
Number of bleeding episodes per year [95% CI]	28.7 [21.9, 37.6]	1.9 [1.5, 2.4]
Traumatic bleeding episodes		
Mean (SD)	28.95 (11.320)	2.28 (2.583)
Median	35.12	1.97
Q1, Q3	15.88, 35.84	0.00, 3.81
Min, Max	15.9, 35.8	0.0, 10.0
Number of bleeding episodes per year [95% CI]	28.2 [21.5, 37.0]	2.5 [2.0, 3.0]
Joint bleeding episodes		
Mean (SD)	43.51 (22.544)	2.91 (4.103)
Median	43.31	1.62
Q1, Q3	21.07, 66.16	0.00, 4.87
Min, Max	21.1, 66.2	0.0, 20.5
Number of bleeding episodes per year [95% CI]	47.1 [38.2, 58.2]	3.3 [2.7, 3.9]
Number of subjects with zero treated bleeding episodes (%)	0	21 (26.3)

Abbreviations: CI, confidence interval; max, maximum; min, minimum; N, total number of subjects within a given regimen; Q1, first quartile; Q3, third quartile.

Note: Number of bleeding episodes per year [95% CI] is based on Poisson distribution.

Twenty-one of the 80 subjects (26.3%) had no bleeding episodes requiring treatment with rVIII-SingleChain.

As expected, the observed ABRs across all bleeding types were substantially higher in the 3 subjects on the on-demand regimen (35.1, 78.6 and 86.6 total bleeding episodes per year).

In the subjects receiving prophylaxis, the median observed ABR for total and joint bleeding episodes was higher in the ≥ 6 to < 12 year age group than in the 0 to < 6 year age group (total bleeding episodes: 5.11 (Q1,Q3: 2.52, 10.50) vs. 2.12 (Q1,Q3: 0.00, 4.54), respectively; joint bleeding episodes: 2.31 vs. 0.00, respectively) Consistent with this, the percentage of subjects with no treated bleeding episode was lower in subjects ≥ 6 to < 12 years (15.6%) than in subjects 0 to < 6 years of age (40.0%). The median AsBR was 0.00 (Q1,Q3: 0.00, 1.46) in subjects 0 to < 6 years of age and 0.00 (Q1,Q3: 0.00, 3.20) in subjects ≥ 6 to < 12 years.

A summary of the ABR by prophylaxis regimen for the 74 subjects with an initial dose assignment in the range of 15 to 50 IU/kg is presented in the following Table.

Table 23: Annualized Bleeding Rate by Prophylaxis Regimen (Efficacy Population, Subjects with Initial Dose Assignment of 15 to 50 IU/kg, study 3002)

Summary of Annualized Bleeding Rates for Prophylaxis
(Efficacy Population, Inital Dose Assignment 30-50 IU/kg)

	Three Times Per Week (N=15)	Two Times Per Week (N=24)
Total Bleeds		
n	15	24
Mean (SD)	4.25 (6.115)	4.54 (4.751)
Median	1.63	2.87
Q1, Q3	0, 6.94	2.01, 5.39
Min, Max	0, 17.5	0, 21.7
Number of bleeds per year (95% CI)	3.9 (2.7, 5.8)	4.5 (3.5, 5.9)
Spontaneous Bleeds		
n	15	24
Mean (SD)	1.43 (2.514)	0.45 (1.183)
Median	0	0
Q1, Q3	0, 2.50	0, 0
Min, Max	0, 7.2	0, 5.1
Number of bleeds per year (95% CI)	1.4 (0.7, 2.7)	0.4 (0.2, 1.0)
Traumatic Bleeds		
n	15	24
Mean (SD)	2.06 (3.129)	2.70 (2.200)
Median	0	2.56
Q1, Q3	0, 4.81	1.58, 3.81
Min, Max	0, 10.0	0, 8.7
Number of bleeds per year (95% CI)	1.9 (1.1, 3.3)	2.7 (1.9, 3.8)
Joint Bleeds		
n	15	24
Mean (SD)	2.83 (4.324)	2.48 (3.698)
Median	0	1.75
Q1, Q3	0, 5.07	0, 2.79
Min, Max	0, 12.5	0, 15.2
Number of bleeds per year (95% CI)	2.7 (1.7, 4.3)	2.5 (1.8, 3.6)
Number of subjects with zero treated bleeds		
n (%)	7 (46.7)	3 (12.5)

Note: [1] Number of bleeds per year (95% CI) based on a Poisson distribution.

The median observed ABR for total bleeding episodes was 2.30 (Q1,Q3: 0.00, 11.58) with the 3-times-weekly regimen and 4.37 (Q1,Q3: 2.31, 7.24) with the 2-times-weekly regimen. The median observed AsBR for spontaneous bleeding episodes was 0.00 (Q1,Q3: 0.00, 3.03) for the 3-times-weekly regimen and 0.00 (Q1,Q3: 0.00, 2.08) for the 2-times-weekly regimens. The number of subjects receiving prophylaxis every second day or at other regimens was too small for conclusive comparisons.

The percentage of subjects with no treated bleeding episodes was higher with the 3-times-weekly regimen (37.5% of subjects) than with the 2-times-weekly regimen (15.0%).

Table 24: Annualized Bleeding Rate by Age Group and rVIII-SingleChain Regimen for Total and Spontaneous Bleeding Episodes (Efficacy Population, study 3002)

	On-demand regimen	Prophylaxis regimen
Age group 0 to < 6 years	**(N = 0)**	**(N = 35)**
Total bleeding episodes		
Mean (SD)	N/A	3.00 (3.932)
Median	N/A	2.12
Q1, Q3	N/A	0.00, 4.54
Min, Max	N/A	0.0, 17.0
Number of bleeding episodes per year [95% CI]	N/A	3.0 [2.3, 4.0]
Spontaneous bleeding episodes		
Mean (SD)	N/A	0.81 (1.499)
Median	N/A	0.00
Q1, Q3	N/A	0.00, 1.46
Min, Max	N/A	0.0, 5.4
Number of bleeding episodes per year [95% CI]	N/A	0.9 [0.5, 1.5]
Number of subjects with zero treated bleeding episodes (%)	0	14 (40.0)
Age group ≥ 6 to < 12 years	**(N = 3)**	**(N = 45)**
Total bleeding episodes		
Mean (SD)	66.77 (27.702)	6.94 (6.047)
Median	78.56	5.11
Q1, Q3	35.12, 86.62	2.52, 10.50
Min, Max	35.1, 86.6	0.0, 23.7
Number of bleeding episodes per year [95% CI]	71.5 [60.3, 84.8]	7.4 [6.3, 8.6]
Spontaneous bleeding episodes		
Mean (SD)	24.83 (22.191)	2.39 (3.607)
Median	31.76	0.00
Q1, Q3	0.00, 42.73	0.00, 3.20
Min, Max	0.0, 42.7	0.0, 14.0
Number of bleeding episodes per year [95% CI]	28.7 [21.9, 37.6]	2.6 [2.0, 3.3]
Number of subjects with zero treated bleeding episodes (%)	0	7 (15.6)

Abbreviations: CI, confidence interval; max, maximum; min, mimimum; N, total number of subjects within a given regimen; N/A, not applicable; Q1, first quartile; Q3, third quartile.

Note: Number of bleeding episodes per year [95% CI] is based on Poisson distribution.

Consumption

A summary of the consumption of rVIII-SingleChain during routine prophylaxis is presented for the 2 most frequent prophylaxis dosing regimens (2- and 3-times-weekly) as well as for all prophylaxis dosing frequencies.

Table 25: Consumption of rVIII-SingleChain during Routine Prophylaxis (Efficacy Population, study 3002)

	2 times per week (N = 43)	3 times per week (N = 24)	All frequencies (N = 80)
Total number of prophylaxis injections	2354	1519	4524
Prophylaxis consumption [1] per subject per month (IU/kg)			
Mean (SD)	325 (100.9)	439 (122.0)	359 (124.2)
Median	320	409	342
Min, Max	179, 547	280, 717	71, 717
Prophylaxis consumption [1] per subject per year (IU/kg)			
Mean (SD)	3902 (1210.6)	5266 (1464.0)	4312 (1490.9)
Median	3836	4913	4109
Min, Max	2142, 6560	3356, 8601	848, 8601
Prophylaxis IU per month [1]			
Mean (SD)	8967 (4920.1)	11808 (5929.0)	9728 (5778.6)
Median	7822	9688	7810
Min, Max	3853, 28791	3560, 23165	870, 28955
Total number of all injections	2590	1704	5088
Total consumption [2] per subject per month (IU/kg)			
Mean (SD)	361 (109.3)	502 (148.3)	414 (180.2)
Median	343	456	378
Min, Max	185, 580	307, 873	153, 1394
Total consumption [2] per subject per year (IU/kg)			
Mean (SD)	4338 (1311.9)	6022 (1779.6)	4974 (2163.0)
Median	4117	5469	4541
Min, Max	2223, 6965	3687, 10473	1839, 16727
Total IU per month [2]			
Mean (SD)	9997 (5378.7)	13964 (8302.9)	11460 (8287.8)
Median	9080	11217	9028
Min, Max	4303, 30373	3689, 34395	1885, 51575

Abbreviations: IU, international units; max, maximum; min, minimum; N, total number of subjects (overall or within a given regimen).

Notes:

[1] Prophylaxis consumption includes those injections recorded as administered for "routine / prophylaxis".

[2] Total consumption also includes those injections recorded as administered for "bleeding event", "surgery", "post surgery", "prevention prior to activity", and "additional treatment".

[3] The remaining 13 subjects were on other prophylaxis regimens (ie, every second day or other frequencies).

Across all prophylaxis regimens, the median prophylaxis dose per subject was 342 IU/kg per month and 4109 IU/kg per year. Despite the protocol allowing for higher doses up to 50 IU/kg in less frequent dosing regimens, doses prescribed per injection were not higher with the 2-times-weekly than with the 3-times-weekly regimen, resulting in a 30% overall lower consumption on the 2-times-weekly regimen.

Since subjects on prophylaxis had a low number of bleeding episodes and administered only few preventative / additional doses, the total consumption of rVIII-SingleChain (ie, prophylaxis doses plus doses to treat bleeding episodes and preventative / additional doses) was not substantially higher than the consumption for prophylaxis alone.

Overall, there were no relevant differences between the age groups in consumption of rVIII-SingleChain.

- **Ancillary analyses**

Not applicable.

- **Summary of main efficacy results**

The following table summarises the efficacy results from study 3002 supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 26: Summary of Efficacy for trial 3002

Title: A Phase III Open-label Pharmacokinetic, Efficacy and Safety Study of rVIII-SingleChain in a Pediatric Population with Severe Haemophilia A			
Study identifier	CSL627_ 3002		
Design	This is an international, multicenter, open-label study to assess the efficacy, safety, and PK profile of rVIII-SingleChain in pediatric patients with severe haemophilia A (FVIII concentration <1%). Subjects received either on-demand or prophylaxis treatment with rVIII-SingleChain at a dose to be determined by the investigator. Haemostatic efficacy was assessed by the subject/caregiver and the investigator who assessed overall efficacy by a 4-point scale.		
	Duration of main phase:	1 year, 5 months	
	Duration of Run-in phase:	NA	
	Duration of Extension phase:	NA	
Hypothesis	The primary objective of the study was to evaluate the efficacy of rVIII-SingleChain in the treatment of major and minor bleeding events based on the investigator's 4-point assessment scale in a pediatric population.		
Treatments groups	rVIII-SingleChain Prophylaxis	Subjects receiving routine prophylaxis treatment were initially treated with 15-50 IU/kg of rVIII-SingleChain every 2nd day or 2 to 3 times per week, or at the investigator's discretion, based upon available PK data, the FVIII treatment regimen used before enrollment and/or the subject's bleeding phenotype. The dose or dosing frequency may have been adjusted if necessary. Preventative and additional doses of rVIII-SingleChain were allowed; data from such doses are included in the analysis of 'Consumption of rVIII-SingleChain' end points. "Preventative dose" was a dose taken before an activity or a minor procedure to prevent or minimize a bleeding episode and "additional dose" was a dose taken beyond the need to control hemostasis.	
	rVIII-SingleChain On-Demand	Subjects assigned to the on-demand treatment regimen treated themselves, or were treated by a caregiver/guardian, as needed for any bleeding episode and did not receive routine assigned infusions. Preventative and additional doses of rVIII-SingleChain were allowed; data from such doses are included in the analysis of 'Consumption of rVIII-SingleChain' end points. "Preventative dose" was defined as a dose taken before an activity or a minor procedure to prevent or minimize a bleeding episode, and "additional dose" was defined as a dose taken beyond the need to control hemostasis.	

	Efficacy Population	The Efficacy Population consisted of all subjects who received at least 1 dose of rVIII-SingleChain as part of either a routine prophylaxis or on-demand regimen during the study. One subject was excluded from the efficacy population because of a pre-existing inhibitor to FVIII (confirmed by reexamination of a screening sample initially reported as negative due to laboratory error).		
Endpoints and definitions	Primary	Treatment Success	Rate of treatment success where treatment success of a bleeding episode is defined as a rating of "excellent" or "good" based on the investigator's overall clinical assessment of haemostatic efficacy (using a 4-point scale of excellent, good, moderate or poor/no response) on the on-demand and prophylaxis regimens combined. The rate of success was based on the number of treated bleeding events; there were 347 treated bleeding events in the Efficacy Population	
	Secondary	Inhibitor formation to FVIII	The number of subjects who develop inhibitors to rVIIISingleChain, defined as a rVIII-SingleChain antibody titer of at least 0.6 Bethesda Units (BU) per mL after receiving study drug	
	Secondary	Annualized bleeding rate	The annualized bleeding rate was defined as the number of bleeding episodes requiring treatment divided by the efficacy evaluation period in days, x 365.25, and is presented separately for the on-demand regimen and the prophylaxis regimens	
	Secondary	Percentage of Bleeding Episodes (BE) Requiring 1, 2, 3 or > 3 Infusions of rVIII-SingleChain to Achieve Hemostasis	Percentage of bleeding episodes requiring 1, 2, 3 or > 3 infusions of rVIII-SingleChain to achieve hemostasis. The denominator includes all treated bleeding episodes	
	Secondary	Consumption of rVIII-SingleChain	IU/kg per subject per month, per year, per Bleeding Event (BE), number of infusions per subject per month, and number of infusions per subject per year	
Database lock	21-Sep-15			
Results and Analysis				
Analysis description	Primary Analysis			
Analysis population and time point description	Efficacy			
Descriptive statistics and estimate	Treatment group	rVIII-SingleChain On-Demand	rVIII-SingleChain Prophylaxis	Efficacy Population
	Number of subjects	3	80	83

variability	Treatment Success [%, (95% CI)]	NA	NA	96.3 (91.3 to 98.4)
	Inhibitor formation to FVIII (subjects)	0	0	0
	ABR for Total Bleeds [Treated bleeding episodes per year, Median (Inter-Quartile Range)]	78.56 (35.12 to 86.62)	3.69 (0.00 to 7.20)	NA
	BE Requiring 1 infusion (Percentage of bleeding episodes)	NA	NA	85.9
	BE Requiring 2 infusions (Percentage of bleeding episodes)	NA	NA	9.8
	BE Requiring 3 infusions (Percentage of bleeding episodes)	NA	NA	2.3
	BE Requiring >3 infusions (Percentage of bleeding episodes)	NA	NA	2.0
	Consumption of rVIII-SingleChain per subject per month [IU/kg per subject per month, Median (Full Range)]	202 (126 to 231)	378 (153 to 1394)	NA
	Consumption of rVIII-SingleChain per subject per year [IU/kg per subject per year, Median (Full Range)]	2429 (1508 to 2771)	4541 (1839 to 16727)	NA
	Consumption of rVIII-SingleChain per subject per BE [IU/kg per event, Median (Full Range)]	25.9 (21 to 78)	37.0 (16 to 282)	NA
	Consumption: Number of infusions per subject per month (on-demand) [number of infusions per subject per month, Median (Full Range)]	7.58 (5.1 to 7.7)	NA	NA
	Consumption: Number of infusions per subject per year [number of infusions per subject per year, Median (Full Range)]	90.95 (60.9 to 92.3)	NA	NA

2.6. Clinical studies in special populations

No patients above 65 years of age were included in the development programme.

2.7. Analysis performed across trials (pooled analyses AND meta-analysis)

Not applicable.

2.7.1. Discussion on clinical efficacy

Design and conduct of clinical studies

Efficacy of rFVIII-SingleChain was mainly evaluated in two Phase 3 studies: Study 1001 in adults and adolescents ≥12 years of age and Study 3002 in children <12 years of age, which both are completed. Moreover, a currently ongoing extension study (3001) is being conducted, where around 60% of the subjects from the Phase 3 studies had been enrolled by the data cut-off date 29 May 2015.

Overall, conduct of the clinical investigation of rFVIII-SingleChain follows the EMA Guideline on the clinical investigation of factor VIII products (EMA/CHMP/BPWP/144533/2009): A sufficient number of previously treated patients with severe haemophilia A out of the relevant age groups were followed for at least 50 EDs, with also a sufficient number of subjects ≥12 years who were followed for at least 6 months for assessment of rFVIII-SingleChain efficacy in prophylaxis. Very limited information about the efficacy and safety are available from patients > 60 years. The requested number of rFVIII-SingleChain response assessments during (major) surgical procedures was satisfied. All subjects in the rVIII-SingleChain studies had a confirmed FVIII level of <1% documented in the source data (inclusion criteria) and with that, a confirmed diagnosis of severe haemophilia A. Therefore efficacy results in relation to dose consumption are not biased by the baseline level of FVIII.

Endpoints chosen are regarded appropriate for efficacy assessment of rFVIII-SingleChain treatment and also in line with the current FVIII Guidance.

The assessment of bleeding episodes such as spontaneous or traumatic was always documented in the database. Spontaneous annualized bleeding rate has been introduced as an efficacy parameter. Such rate might serve as a parameter for efficacy of prophylaxis as it is “countable” and represents an easy-to-understand number. However, lack of definitions for a “bleed”, for “spontaneous” or “traumatic” nature of such bleed and individual evaluation factors are considered to be highly challenging when comparing numbers. Furthermore, comparison of ABR while on on-demand versus on prophylaxis-regimen is considered to be of highly restricted value: On-demand therapy mainly represents severity of haemophilia (overall bleeding frequency) and not efficacy of a certain product. On the other hand, total ABR while on prophylaxis ideally should aim at zero bleeds and should be amended by documentation of exceptional challenges during the evaluation period.

rFVIII-SingleChain efficacy was investigated in treatment and prevention of bleeds, routine prophylaxis and perioperative management by assessment of treatment response rating, rFVIII-SingleChain consumption and determination of (annualised) bleeding rates. The treatment of bleedings in the on-demand setting is regarded as a different situation e.g. localization, severity and frequency of the bleed. Therefore, in this non-randomized study, subjects were able to choose whether to receive on-demand or prophylaxis treatment. It is accepted because randomization of a subject from their current prophylactic regimen to on-demand treatment would be considered unethical. Presentation of results of response rating was done for all kinds of bleeding episodes. As requested, the response assessment stratified by severity of the bleeding episodes has been provided by the applicant. Median dose per injection (IU/kg/injection) and median total dose (IU/kg/bleed) in minor and moderate bleeds are comparable between groups. However, in major bleeds both median dose per injection (48.9 vs. 31.6) and median total dose (50.5 vs. 31.8) were higher in study 3002. This discrepancy seems to attribute to the fact that in children those bleeds stratified into the major category were treated with a

higher dose. Overall treatment success varied from 88.9% (Study 3002, major bleeds) to 100% (Study 1001, minor bleeds).

The statistical methods applied are considered appropriate in general.

Efficacy data and additional analyses

Study1001

The efficacy population of Study 1001 comprised 173 subjects ⩾12 to ⩽65 years (27 subjects on an on-demand regimen, 146 subjects on a prophylaxis regimen) exposed to rVIII-SingleChain for 14,306 EDs and treating 848 bleeding episodes overall.

Demographic and baseline characteristics of study subjects reflect the typical population of patients with haemophilia A. The Applicant has chosen to exclude subjects older than 65 years. Thus, efficacy data in this subpopulation is missing completely. Therefore, the SmPC does list data in this subpopulation as missing..

In Study 1001, adult and adolescent subjects receiving prophylaxis (N=146) had a median ABR of 1.14 (Q1,Q3: 0.0, 4.2) bleeding episodes/year and a median AsBR of 0.00 (Q1,Q3: 0.0, 2.4) bleeding episodes/year. Both values were significantly lower than the median ABR and AsBR in subjects receiving on-demand treatment (N=27); 19.64 (Q1,Q3: 6.2, 46.5)and 11.73 (Q1,Q3: 2.8, 36.5) bleeding episodes/year, respectively. Based on a Poisson model, this represents a reduction in mean ABR and AsBR by ⩾ 90% with prophylaxis compared to on-demand.

The majority of spontaneous bleeding episodes that required treatment were located in the joint, followed by the muscle and other locations, which is consistent with the nature of the disease (severe haemophilia).

In general, the annualised bleeding rates are regarded to be in an acceptable range. It is recognized that one subject who achieved a prophylactic treatment regimen had 40 spontaneous bleeding episodes per year. The Applicant has thoroughly discussed all cases of subjects who achieved a prophylactic treatment regimen with more than 5 bleeding episodes. Common contributing factors, significant joint inflammation or a more severe bleeding phenotype explain the observed higher number of bleeds.

In Study 1001, there were no differences in ABR and AsBR between the 2-times weekly and 3-times-weekly regimens, neither in the initial nor the final dose assignment analysis. The median ABR and AsBR were both 0.00 in the 46 subjects on a 2-times-weekly prophylaxis regimen with a dose of 20 to 50 IU/kg and 1.53 and 0.00 in the 77 subjects on a 3-times-weekly regimen with a dose of 20 to 50 IU/kg.

In adults/adolescents in Study 1001 on a 3-times-weekly prophylaxis regimen, the median starting dose was 30 IU/kg per injection; subjects dosed 2 times weekly had a median starting dose of 35 IU/kg per injection. rVIII-SingleChain consumption across all regimens was 4,283 IU/kg/year (median) and 4494 IU/kg/year (mean). Presentation of consumption of FVIII follows the Clinical Guideline. The presented consumption-data support efficacy of rFVIII-SingleChain, overall.

A dose of 20 to 50 IU/kg administered 2 to 3 times weekly is proposed for prophylaxis. The Applicant was asked to provide individual patient data listings of injections reflecting dose/kg and date of application for each patient as well as stratification in age groups. The additional data, as presented by the applicant, were regarded sufficient to support the dose recommendations in the adolescent and adult population.

As requested the Applicant provided the overall prophylaxis consumption as “total dose administered per subject per month (IU/kg)” including preventative doses (Prevention Prior to Activity) as well as doses for

'Bleeding Event', 'Surgery', 'Post Surgery', and 'Additional Treatment'. Additional details why the preventative dose was administered were not documented; therefore an individual summary of reasons for administration is not available. Instead, the applicant presented a compliance calculation. This was accepted.

In subjects ⩾12 to ⩽65 years in Study 1001, treatment success (ie, an investigator haemostatic efficacy rating of "excellent" or "good") was documented in 92.3% of all treated bleeding episodes assessed in the on-demand and prophylaxis regimens combined.

The Applicant provided information regarding number of infusions for treatment of a bleeding episode. In Study 1001 in adults/adolescents, 80.9% of bleeding episodes were controlled with 1 injection, 12.6% with 2 injections, and 3.4% with 3 injections of rVIII-SingleChain which is regarded as a good and sufficient response. In summary, breakthrough bleeding episodes on prophylaxis were controlled with 1 or 2 injections of rVIII-SingleChain in 90.7% of events in adult/adolescent subjects.

Overall, the success rates of the treatment of bleeds using rVIII-SingleChain are in an acceptable range. In no patients the haemostatic response was rated as poor. More bleeding episodes per subject were reported in the on-demand regimen than in the prophylaxis regimen, in line with expectations for the treatment modalities.

There are 16 surgeries among the 13 subjects in the Surgical Population. A sufficient number of major surgeries were evaluated for rFVIII-SingleChain efficacy. The provided data sufficiently show haemostatic effect of rVIII-SingleChain during surgery and fulfil the requirements of the guideline. However, no pediatric subjects <18 years of age were included in the surgery population. It is considered appropriate to mention this in the SmPC.

Study 3002

The efficacy of rVIII-SingleChain in treatment of bleeding episodes and routine prophylaxis was assessed in 83 subjects < 12 years of age with haemophilia A.

It should be noted that only 3 subjects were assigned to an on-demand regimen, and that no formal comparisons of ABR between on-demand and prophylaxis regimens were performed.

There were a total of 389 bleeding episodes in the 83 subjects in the Efficacy Population. Of these, 347 episodes in 62 subjects required treatment with rVIII-SingleChain. The number of treated bleeding episodes per subject was higher in the on-demand regimen (total of 132 episodes in 3 subjects) than in the prophylaxis regimen (total of 215 episodes in 59 subjects), as expected with the assigned treatment modality.

Overall, the most frequent location of bleeding episodes was the joint (61.7%), followed by other locations (23.1%), and the muscle (14.4%).

Subjects in the age group ⩾6 to <12 years had more total bleeding episodes (especially a higher proportion of traumatic bleeding episodes). This is considered to a higher level physical activity in the older age group.

Investigator's assessment of haemostatic efficacy in 347 bleeds was "excellent" or "good" in the majority (96.3%) of bleeding episodes which is considered be acceptable. One patient is found for response rated as "poor/none response" and further 12 patients are documented with "moderate response". The applicant was asked to comment. From this further analysis no clinically relevant impact on the final efficacy conclusion from Studies 3002 was identified. For 42 bleeds no FVIII-substitution was required, at all.

Treatment success was documented in 96.3% of all treated bleeding episodes in the on-demand and prophylaxis regimens combined. 85.9% of bleeding episodes were controlled with 1 injection, 9.8% with 2 injections, and 2.3% with 3 injections of rVIII-SingleChain.

The median ABR was higher with the 2-times-weekly regimen than with the 3-times-weekly regimen (medians of 4.37 (Q1,Q3: 2.31, 7.24) and 2.30 (Q1,Q3: 0.00, 2.08), respectively) as well as in the older age group than in the younger age group (medians of 5.11 and 2.12, respectively). The higher ABR in the 2-times-weekly regimen might reflect the higher CL and shorter t1/2 of rVIII-SingleChain in children, but could also be the result of an almost 30% lower weekly consumption in subjects assigned to a 2-times-weekly regimen. The higher ABR in the older age group might reflect a more active population. These findings suggest that higher rVIII-SingleChain doses might be considered for children on a 2-times-weekly prophylaxis regimen, and more frequent injections might be required in active children. The median observed AsBR for spontaneous bleeding episodes was 0.00 (Q1,Q3: 0.00, 3.03) for the 3-times-weekly regimen and 0.00 (Q1,Q3: 0.00, 2.08) for the 2-times-weekly regimens.

According to PK simulations in the group < 6 years old receiving a dose regimen 30 IU/kg twice weekly (either day 0 and 3.5 or day 0 and 4) only 54.7% and 46.2% remained at a trough level > 1% whereas in the group >12 years old 73.7% and 64.2 % remained. If only these PK simulations are taken into account, this percentage is considered to be too low to support the twice weekly 30 IU/kg regimen in the paediatric population.

It was requested that the Applicant provide the ABR and AsBR for subjects on 2- and 3-times- weekly regimens stratified by age group and stratified in dose range. In the age groups 0 to < 6 years and 6 to <12 years the ABR and AsBR was higher in the <30 IU/kg range. Therefore, the Applicant recommends a starting dose for children < 12 years of age on prophylaxis of 30 to 50 IU/kg 2 or 3 times weekly taking also into account the presented PK simulations. In principle this dose modification was endorsed, but the dose regimen 30 IU/kg 2 times weekly was still considered questionable especially in the younger children population. However, after further detailed analysis of stratified data, it is agreed that in children dosage regimens above 30 IU/kg are sufficient to achieve ABR and AsBRs comparable with other recombinant FVIII products. Thus, the SmPC recommendation of starting dose for children of 30 to 50 IU/kg 2 or 3 time weekly is considered acceptable.

Across all prophylaxis regimens, the median prophylaxis dose per subject was 342 IU/kg per month and 4109 IU/kg per year. The overall annual consumption across all regimens was very similar to the values in Study 1001 (rVIII-SingleChain consumption: 4,283 IU/kg/year [median] and 4,494 IU/kg/year [mean]).

The respective values in adults and children are reflected in the SmPC, as reference for efficacy in prophylaxis.

2.7.2. Conclusions on the clinical efficacy

Efficacy has been analysed for prophylaxis, on-demand treatment, treatment of breakthrough bleeding episodes and prophylaxis for surgical procedures. Study designs, selection and number of patients, assessment tools and results are in general adequate for supporting efficacy of the rVIII-SingleChain.

In summary, rVIII-SingleChain is an effective FVIII replacement therapy which is considered to be suitable for the control and prevention of bleeding episodes, for use as routine prophylaxis with treatment intervals of 2 to 3 times weekly, and for perioperative prophylaxis in adult and adolescent patients and children with haemophilia A.

2.8. Clinical safety

Patient exposure

Table 27 Exposure to rVIII-SingleChain in Clinical Studies (Safety Populations, Study 1001 and Study 3002, and Enrolled Population, Study 3001)

	Study 1001 (N = 174)	Study 3002 (N = 84)	Study 3001[a,b] (N = 154)
Exposure days[c]			
Mean (SD)	82.2 (61.35)	62.4 (24.73)	145.2 (68.69)
Median (min, max)	63.5 (1, 395)	58.5 (4, 142)	135.5 (22, 496)
< 50 EDs, n (%)	54 (31.0)	19 (22.6)	2 (1.3)
≥ 50 EDs, n (%)	120 (69.0)	65 (77.4)	152 (98.7)
≥ 100 EDs, n (%)	52 (29.9)	8 (9.5)	109 (70.8)
Total number of EDs	14306	5239	22362
Study duration, days			
Mean (SD)	258.8 (163.52)	183.5 (61.16)	162.5 (56.97)
Median (min, max)	219.0 (5, 758)	171.0 (30, 346)	176.0 (1, 457)
Study duration, months			
Mean (SD)	8.5 (5.37)	6.0 (2.01)	5.3 (1.87)
Median (min, max)	7.2 (0, 25)	5.6 (1, 11)	5.8 (0, 15)
Total subject-years[d]	122.7	42.2	68.1
Number of rVIII-SingleChain injections per subject			
Mean (SD)	83.9 (61.96)	63.3 (25.77)	NR
Median (min, max)	67.0 (1, 395)	59.0 (4, 145)	NR
Total number of rVIII-SingleChain injections	14592	5313	NR

Abbreviations: ED, exposure day; max, maximum; min, minimum; N, total number of subjects in the Safety Population of a given study (or Enrolled Population for Study 3001); n, number of subjects with a given criterion; NR, not reported; rVIII-SingleChain, recombinant single-chain factor VIII.

[a] Interim exposure data for Study 3001 up to 29 May 2015.

[b] For Study 3001, EDs are counted from the first day of exposure to rVIII-SingleChain in the pivotal studies.

[c] An ED is any day that the subject receives an injection of rVIII-SingleChain regardless of the number of injections on that day.

[d] Subject-years = ([date of last visit] – minimum[date of first rVIII-SingleChain dose, date of Day 1 of the treatment period] + 1]) / 365.25

The overall safety population comprises a total of 258 unique individuals (174 from Study 1001 and 84 from Study 3002) who received at least 1 dose of rVIII-SingleChain, with a total of 19,545 EDs.

In Study 1001, 120 of the 174 adolescent/adult subjects achieved ≥ 50 EDs, and 52 subjects ≥ 100 EDs. In Study 3002, 65 of the 84 pediatric subjects achieved ≥ 50 EDs (thereof 27 subjects 0 to < 6 years and 38 subjects ≥6 to < 12 years), and 8 subjects ≥ 100 EDs.

In Extension Study 3001, an additional 8,873 EDs have been documented in the 154 subjects as of the cutoff date for this submission (132 adults and adolescents from Study 1001 and 32 children from Study 3002). Combined with the exposure achieved in the pivotal studies, total exposure is 28,418 EDs in the clinical program, with 109 subjects achieving ≥ 100 EDs.

Adverse events

In Study 1001, 113 subjects experienced a total of 292 treatment-emergent AEs (TEAEs) in a total of 14,592 injections and 14,306 EDs with rVIII-SingleChain. The 3 most common TEAEs were nasopharyngitis, arthralgia and headache. Of the 10 TESAEs reported in this study, 1 was assessed as related by the investigator; this was an event of hypersensitivity that could be controlled by administration of steroids and antihistamines allowing hospital discharge on the day of the event, and the subject remained on rVIII-SingleChain treatment and tolerated it well. No subject withdrew from the study due to a TEAE.

Table 28: Related treatment-emergent adverse events by system organ class and preferred term (safety population, study 1001)

System Organ Class Preferred Term	No. (%) of subjects	No. of events
Related TEAEs	13 (7.5)	19
General disorders and administration site conditions	**4 (2.3)**	**5**
Chills	1 (0.6)	2
Feeling hot	1 (0.6)	1
Injection site pain	1 (0.6)	1
Pyrexia	1 (0.6)	1
Nervous system disorders	**3 (1.7)**	**3**
Dizziness	2 (1.1)	2
Paraesthesia	1 (0.6)	1
Skin and subcutaneous tissue disorders	**3 (1.7)**	**3**
Erythema	1 (0.6)	1
Pruritus	1 (0.6)	1
Rash	1 (0.6)	1
Immune system disorders	**2 (1.1)**	**3**
Hypersensitivity	2 (1.1)	3
Musculoskeletal and connective tissue disorders	**1 (0.6)**	**4**
Arthralgia	1 (0.6)	3
Joint range of motion decreased	1 (0.6)	1
Investigations	**1 (0.6)**	**1**
Drug specific antibody present	1 (0.6)	1

TEAE, treatment-emergent adverse event.

Notes:

[1] Table presents number and percentage of subjects [n (%)] with at least 1 specified event and number of specified events (E).

[2] Percentages are based on the number of subjects in the respective group.

[3] Events with onset date during the surgical period are excluded from TEAEs.

[4] Adverse events were coded using MedDRA Version 14.1.

[5] For the subject with the event of 'drug specific antibody present', the presence of an anti-drug antibody (ADA) was recorded as a TEAE in error by the investigator; ADAs were only to be collected as a laboratory investigation

In Study 3002, 64 subjects experienced a total of 183 TEAEs in a total of 5,313 injections and 5,239 EDs with rVIII-SingleChain. The most common TEAEs in subjects 0 to < 12 years were nasopharyngitis, arthralgia, cough, and headache. One TEAE (drug hypersensitivity) was assessed as related to rVIII-SingleChain. Of the 11 TESAEs, none were assessed as related by the investigator. One subject withdrew from the study due to a non-related TEAE of hip arthralgia.

In Study 3001, 91 AEs in 48 subjects have been reported, with 1 of them (drug hypersensitivity, non-serious) assessed as related to rVIII-SingleChain by the Sponsor. The most common AE in this study was nasopharyngitis. Of the 6 SAEs, none was assessed as related by the investigator. Two subjects withdrew from the study due to AEs .

The PTs were categorized as undesirable effects after careful review [by the Sponsor] of all TEAEs. Those include TEAEs occurring in ⩾ 2 subjects, TEAEs considered by the investigator as related to rVIII-SingleChain, and TESAEs from Studies 1001, 3002, and 3001 (up to 29 May 2015). In addition, in Study 1001, 4 subjects turned positive for non-inhibitory ADAs, but only 2 of these subjects remained positive at the End-of-study visit. In Study 3002, 10 subjects turned positive for non-inhibitory ADAs; 7 of these subjects remained positive at the End-of-study visit.

The SOC with the highest incidence of AEs was Infections and infestations (38 [45.2%] subjects). The most commonly reported AEs (> 5% of subjects) were nasopharyngitis (14 [16.7%] subjects, 15 events), arthralgia (8 [9.5%] subjects, 8 events), cough (7 [8.3%] subjects, 10 events), headache (7 [8.3%] subjects, 9 events), head injury (5 [6.0%] subjects; 6 events), and pyrexia (5 [6.0%] subjects; 5 events).

Table 29 Undesirable Effects by System Organ Class and Preferred Term (Safety Populations, Studies 1001 and 3002, and Enrolled Population, Study 3001)

System organ class Preferred term	Number (%) of subjects (N = 154)[a]	Number of events
Any AE	**48 (31.2)**	**91**
Infections and infestations	**25 (16.2)**	**30**
Nasopharyngitis	8 (5.2)	9
Upper respiratory tract infection	4 (2.6)	6
Tonsillitis	3 (1.9)	3
Injury, poisoning and procedural complications	**13 (8.4)**	**22**
Laceration	3 (1.9)	3
Contusion	2 (1.3)	3
Tongue injury	2 (1.3)	2
Musculoskeletal and connective tissue disorders	**5 (3.2)**	**5**
Haemophilic arthropathy	2 (1.3)	2
Eye disorders	**2 (1.3)**	**2**
Conjunctivitis	2 (1.3)	2

Abbreviations: AE, adverse event; N, number of subjects in the Enrolled Population.

[a] Interim data up to 29 May 2015.

Serious adverse event / deaths / other significant events

There were no deaths in Studies 1001, 3002, or 3001.

In Study 1001, 7 (4.0%) subjects experienced 9 TESAEs, of which 5 were severe, including 1 (hypersensitivity) that was severe and considered by the investigator to be related to the study drug. All subjects fully recovered.

In Study 3002, 9 (10.7%) subjects had 11 TESAEs, none of which were considered related to rVIII-SingleChain, and all subjects fully recovered.

In study 3001, 5 (3.2%) subjects had 6 SAEs none of which were considered by the investigator as related to the study drug. All SAEs were reported as resolved as of 29 May 2015.

Laboratory findings

Hematology and clinical chemistry data are only presented for the completed Studies 1001 and 3002; the results for ongoing Study 3001 will be provided in the final CSR for Study 3001.

Hematology

In Studies 1001 and 3002, the mean changes across all hematology parameters were small from baseline to the End-of-study visit and from baseline to the lowest or highest value after baseline. For any given parameter, there were never more than 2 (1.1%) subjects in Study 1001 and 2 (2.4%) subjects in Study 3002 who reported a clinically significant abnormal result at any visit (References have been provided).

In Study 1001, 3 subjects had TEAEs of anemia, none of which were considered related to rVIII-SingleChain by the investigator. For 2 of these subjects, the anemia was non-serious and mild, and was reported as not resolved: for 1 of these subjects, anemia was already present in the subject's medical history and the other subject was treated with iron. For the third subject, the anemia was serious and severe, and led to hospitalization but was reported as resolved.

In Study 3002, 6 subjects had TEAEs of anemia, none of which were considered related to rVIII-SingleChain by the investigator. In 5 of the 6 subjects, the anemia TEAEs were non-serious and mild or moderate in intensity. Two of these events were iron deficiency anemia, and 3 events were treated with iron. Except for 2 events (1 resolving and 1 not resolved), all anemia TEAEs were reported as resolved at the last available assessment. In the remaining subject, 3 separate TESAEs of anemia were reported. Respective references and narratives have been provided.

Clinical Chemistry

In Studies 1001 and 3002, the mean changes across all biochemistry parameters were small from baseline to the End-of-study visit and from baseline to the lowest or highest value after baseline.

In Study 1001, for any given parameter, there were never more than 2 (1.1%) subjects who reported a clinically significant abnormal result. A total of 9 (5.2%) subjects experienced 13 TEAEs in the SOC of Investigations. This included 1 subject with the TEAE of drug-specific antibody present; this event was reported as a TEAE by mistake, as antibody findings were not meant to be reported as AEs. In the other 8 subjects, other than liver function test abnormal (2 [1.1%] subjects, 1 event each), none of the investigation PTs were experienced by more than 1 subject. None of these were assessed as related TEAEs by the investigator.

In Study 3002, no subjects reported treatment-emergent abnormal values assessed as clinically significant by the investigator. No consistent trends or patterns regarding changes / abnormal values in renal or hepatic parameters were identified. Cases of marginally elevated liver enzyme or elevated

bilirubin levels primarily occurred in subjects with already elevated levels at Screening / Day 1 or were isolated cases that did not reoccur. Respective references have been provided.

Viral safety

The viral safety of rVIII-SingleChain final product is assured by the combination of virus testing of the expression system, the use of animal component-free media in the purification and formulation process, and the incorporation of dedicated virus removal / inactivation steps. The outcome of this comprehensive strategy is a final product that has a high level of virus safety.

Viral safety retention samples were to be analyzed only if there is a suspicion of a viral infection. None of the subjects who participated in Study 1001, Study 3002, or Study 3001 had an event that warranted testing of the stored study blood samples for the presence of viral markers; the results for ongoing Study 3001 will be provided in the final CSR. Respective references have been provided.

Vital Signs and Physical Findings

Vital signs and physical findings data are only presented for the completed Studies 1001 and 3002; the results for ongoing Study 3001 will be provided in the final CSR.

No relevant changes in vital signs were observed in Study 1001 (including the Part 3 PK comparison of high and low strength formulations) or Study 3002. Potentially clinically significant changes in vital sign values at the end of Study 1001 were reported for 2 (1.1%) subjects, 1 with high and 1 with low diastolic blood pressure. In Study 3002, most of the potentially clinically significant values were isolated cases, and there was no indication of a clustering of potentially significant vital signs values in a particular age group or treatment modality. References have been provided.

Safety in special populations

No subject >65 years of age has been investigated in any of the clinical trials.

The incidence of TEAEs was similar in subjects ⩾ 12 to < 18 years (10 [71.4%] subjects) and ⩾ 18 to ⩽ 65 years (103 [64.4%] subjects) in Study 1001, and in subjects 0 to < 6 years (28 [80.0%] subjects) and ⩾ 6 to < 12 years (36 [73.5%] subjects) in Study 3002.

The proportion of related TEAEs was also similar in both age groups in Study 1001 (1 [7.1%] subjects ⩾ 12 to < 18 years and 12 [7.5%] subjects ⩾ 18 to ⩽ 65 years) and in both age groups in Study 3002 (no subjects 0 to < 6 years and 1 [2.0%] subject ⩾ 6 to < 12 years).

In the interim data of Study 3001, AEs were reported in 1 (14.3%) subject 0 to < 6 years, 1 (6.7%) subject ≥ 6 to < 12 years, 7 (50.0%) subjects ≥ 12 to < 18 years, and 39 (33.1%) subjects ≥ 18 to ≤ 65 years. The safety profile of rVIII-SingleChain in the 2 age groups (0 to < 18 years and ≥ 18 to ≤ 65 years) was consistent with what was documented in the pivotal studies.

Table 30 Summary of AEs by Age (Safety Populations, Studies 1001 and 3002)

Type of AE	Number (%) of subjects			
	Study 3002		Study 1001	
	0 to < 6 years (N = 35)	≥ 6 to < 12 years (N = 49)	≥ 12 to < 18 years (N = 14)	≥ 18 to ≤ 65 years (N = 160)
AEs	28 (80.0)	36 (73.5)	10 (71.4)	111 (69.4)
TEAEs	28 (80.0)	36 (73.5)	10 (71.4)	103 (64.4)
TEAEs leading to study withdrawal	0	1 (2.0)	0	0
TEAE related to study drug	0	1 (2.0)	1 (7.1)	12 (7.5)
TEAE outcome				
Death related to AE	0	0	0	0
SAEs	4 (11.4)	5 (10.2)	2 (14.3)	6 (3.8)
TESAEs	4 (11.4)	5 (10.2)	2 (14.3)	5 (3.1)
TESAEs related to study drug	0	0	1 (7.1)	0

Abbreviations: AE, adverse event; N, total number of subjects within an age group in the Safety Population of a given study; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event.
Note: For Study 1001, AEs and SAEs include those with an onset date prior to study drug start date and during the surgical period.
Source: Study 1001 CSR, Table 14.3.2.14 and Study 3002 CSR, Table 14.3.1.1

Immunological events

Inhibitors

No subject developed an inhibitor under exposure to rVIII-SingleChain in any of the studies. In study 3002, one paediatric subject with initially negative inhibitor-titre, retrospectively turned out to have had pre-existing inhibitor. A low-titre inhibitor was detected at 4 weeks on AFSTYLA; retrospectively, the negative screening test (central lab) was repeated and found to be positive. Furthermore, this subject had positive ADAs (IgG), initially, experienced a TEAE of hypersensitivity at his second treatment day which required treatment with antihistamines, and the subject experienced a TESAE of bacteraemia with chills and fever for central venous catheter infection. FVIII-substitution-dose was doubled (from 25 to 50 IU/kg 3 times weekly) and inhibitor titre decreased.

The Applicant has developed three methods for determining the anti-rVIII-SingleChain-antibodies. The assays to determine non-inhibitory ADAs have been appropriately validated.

Information with regards to inhibitor development is provided in section 4.4 of the SmPC.

Non-inhibitory Anti-drug Antibodies and Anti-CHO Antibodies

Numbers of subjects with positive tests at baseline or any time post baseline for non-inhibitory ADAs are summarized: A subject's result was counted as positive if the result from ADAs was positive and the test result for either IgG or immunoglobulin M (IgM) was also positive, otherwise the subject's result was counted as negative:

- In Study 1001, 8 subjects entered the study with positive non-inhibitory ADA (IgG and/or IgM) test. Four other subjects entered the study with negative ADA and turned positive for IgG and/or IgM during the study. 2 of them were negative at End-of-study.

- In Study 3002, 10 subjects entered the study with a positive test for non-inhibitory ADAs. Ten other subjects entered the study with negative ADA and turned positive for non-inhibitory ADAs at some point during the study. 3 of them were negative at End-of-study.

- In Study 3001, no further subjects developed an ADA.

Of 14 subjects, who turned positive after being negative for ADAs at baseline, the Applicant is asked to provide information on possible associated clinical symptoms (studies 1001 and 3002) and laboratory findings (especially relation of ADA positivity to lowering of RBC). No positive relationship was found.

The Applicant was asked for the ADA-status of subjects with normocytic anaemia. However, ADAs were negative.

None of the subjects who tested positive for non-inhibitory ADAs experienced TEAEs that, upon medical review, were assessed to be associated with any of the reported non-inhibitory ADAs.

No subject in any of the rVIII-SingleChain clinical studies entered with anti-CHO host cell protein antibodies or developed these during the studies.

Hypersensitivity Reactions

In Study 1001, the predefined search strategy with Standardized MedDRA Queries (SMQs) identified 15 subjects (8.5%) who experienced events that could be considered as symptoms or manifestations of hypersensitivity. 2 subjects (1.1%) experienced hypersensitivity reactions. Upon medical review by the sponsor, events of cough and sneezing were not considered to be associated with hypersensitivity reactions. None of the events identified were considered to be anaphylactic reactions.

6 TEAEs indicative of hypersensitivity were considered to be related to the study drug in 4 of the subjects.

In study 3002, the predefined search strategy with SMQs identified 13 subjects (15.5%) who experienced 19 events that could be considered as symptoms or manifestations of hypersensitivity. All but 1 of these events (hypersensitivity) were considered as unrelated by the investigator. The related event of hypersensitivity was mild in intensity and the dose of rVIII-SingleChain was not changed as a result of this event. Respective subject has been described, above, as case-report of pre-existing inhibitor, ADA-positivity, catheter-related sepsis and hypersensitivity.

In the interim data up to 29 May 2015 for Study 3001, the predefined search strategy with SMQs identified 4 subjects (all in the $\geq$ 18 to $\leq$ 65 year age group) who experienced AEs that were reported as hypersensitivity (eczema, rash pruritic, rash, and drug hypersensitivity). All but 1 of these events (drug hypersensitivity, see below) were considered as unrelated by the investigator and adjudicators.

This subject with hypersensitivity: received 153 EDs in study 1001 and further 65 EDs in study 3001. He experienced a non-serious event of drug hypersensitivity. The event resolved after 1 day. 12 days later, the subject experienced positive re-challenge without the use of premedication and was withdrawn from the study.

Cardiovascular events

In patients with existing cardiovascular risk factors, substitution therapy with factor VIII may increase the cardiovascular risk. There were no such events observed in studies.

Catheter-related complications

If a central venous access device (CVAD) is required, risk of CVAD-related complications including local infections, bacteraemia and catheter site thrombosis should be considered.

Sodium content

This medicine contains up to 7 mg (0.3 mmol) sodium per ml after reconstitution. To be taken into consideration by patients on a controlled sodium diet.

Thromboembolic Events

There were no intravascular TEEs in any of the 3 clinical studies. In Study 3002, 1 subject experienced a TESAE of device occlusion that was identified by the SMQ search “Embolic and thrombotic events”. Furthermore, risk for TEEs in the elderly population remains open, as no subject beyond 64 years of age has been followed in clinical studies, yet.

Local Tolerability

Local tolerability at the injection site was assessed by study subjects on a 5-point scale, and by the investigator using a 5-point scale for erythema and a separate 5-point scale for itching, local pain, or local heat, and by measuring the size of any edema or induration.

In Study 1001, 99.3% of the 13,580 injections assessed by the subjects had no reactions. In Study 3002, 99.4% of the 4,774 injections assessed by the subjects had no reactions. In Study 3001, 99.9% of the 8,587 injections assessed have been reported by the subjects with no reactions. No severe local reactions were reported in any study.

Overdose

Any TEAE associated with $\geqslant$ 2 times the prescribed rVIII-SingleChain dose was considered as an overdose and was to be documented in the electronic case report form and reported to the sponsor within 72 h.

One subject from study 1001 reported a TEAE that was associated with $\geqslant$ 2 times the prescribed rVIII-SingleChain dose. The subject was prescribed between 35-50 IU/kg rVIIISingleChain for a bleeding episode, but was administered 6 vials of Afstyla, which was 111.3 IU/kg. The subject complained of dizziness and feeling hot and itchy after taking the study drug and Tramal. The investigator rated these events as not related to study drug but to the co-administered analgesic. These events were summarized and coded to the PT “adverse drug reaction”, which was mild and not serious.

Effects on ability to drive and use machines

Afstyla has no influence on the ability to drive and use machines.

Safety related to drug-drug interactions and other interactions

Not applicable.

Discontinuation due to adverse events

No subjects were withdrawn from Study 1001 due to AEs.

One subject was withdrawn from Study 3002 due to hip arthralgia.

Two subjects have been withdrawn from Study 3001, due to AEs.

Post marketing experience

Not applicable.

2.8.1. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Exposure and safety were assessed in all subjects who received a dose of rVIII-SingleChain as part of either a PK evaluation, on-demand treatment of bleeding episodes, routine prophylaxis, or perioperative management of bleeding episodes. Safety was assessed based on adverse event (AE) reporting, routine laboratory safety, local tolerability, vital signs, and physical examinations. Important identified and potential risks that are class effects for rFVIII and pdFVIII concentrates include hypersensitivity / anaphylactic reactions, development of inhibitors against FVIII, development of non-inhibitory ADAs and antibodies against CHO host cell proteins.

According to the Guideline on clinical investigation of recombinant factor VIII and IX products, an overall number of 100 individuals is requested for pre-authorisation evaluation of general safety-aspects. At least 50 subjects ≥ 12 years of age should be followed for at least 50 Exposure Days (ED) and at least 50 children <12 years and separated into two age-cohorts of 25 subjects, each, should have reached 50 ED, too. Such requirements have been met by the presented numbers.

Furthermore, 14 adolescents ≥ 12 to < 18 years of age were included into study 1001; 8 of 14 reached > 50 EDs and 2/14 >100 EDs. There is no requirement of specific numbers for adolescents in the clinical guideline.

Standard Laboratory findings and Vital-signs- and Physical-findings-documentation was within expected range.

Numbers of Adverse Events and AE-profile according to SOCs are in line with similar factor concentrates. 26 TESAEs in 21 subjects were documented. Hypersensitivity was the most relevant symptom to be recognized in relation to Afstyla. One patient with pre-existing inhibitor experienced Hypersensitivity, and venous-device complication.

Adverse events of special interest were documented and presented: One low-titre inhibitor retrospectively turned out to be pre-existent. Number of non-inhibitory ADAs did not raise concerns, although the numbers in children are higher than in adults.

The Guideline on clinical investigation of recombinant FVIII and IX products does not request inclusion of elderly subjects. Of note, the clinical safety database of rVIII-SingleChain is limited regarding elderly patients. Although no subject experienced TEE, the constantly growing elderly population is at higher risk for thromboembolism. Experience in geriatric patients (65 years and above) has been included as missing information in the RMP (please see RMP section).

Paediatric subjects have been included into study 1001 (adolescents, 12-<18 years of age), study 3002 (< 12 years of age) and into study 3001 (0-<18years of age).

In addition, Factor VIII monitoring errors due to discrepancy of one-stage versus chromogenic-test-results might not have been identified under clinical trials conditions although potentially a top-up dose might be given, which, while unnecessary, would not result in an additional risk for the affected patient. In healthy individuals, FVIII circulates in plasma at concentrations of 50 to 150 IU/dL. However, FVIII is an acute-phase reactant, and plasma levels in healthy individuals without congenital bleeding disorders rise above 240% in acute situations such as surgery, sports, and infections. While chronically elevated FVIII levels have been identified as a risk factor for thrombosis in patients without congenital bleeding disorders that is not the case for acutely elevated FVIII plasma activity. Given the short plasma half-life of FVIII in haemophilia patients, high doses (even in a prophylaxis regimen) would never reach steady state (ie, chronically elevated levels). Adverse reactions due to dosing errors caused by misinterpretation of monitoring results are not biologically plausible and therefore are not anticipated. To avoid dosing errors due to discrepancy of one-stage versus chromogenic-test-results, a factor 2 for conversion of test results has been introduced.

Additional information in Previously Untreated Patients (PUPs) is crucial in the context of treatment in haemophilia A. Experience of inhibitor formation in PUPs has been included as missing information and development of inhibitors has been included as an important identified risk.

In order to obtain further safety data (on the identified and potential risks described in the RMP below) in prophylaxis, on-demand long-term treatment and in surgery, the company should submit the results of Study 3001, a phase III open label, multicenter, extension study to assess the safety and efficacy of recombinant coagulation Factor VIII (rVIII-SingleChain, CSL627) in subjects with severe haemophilia A.

In order to investigate the hypersensitivity/anaphylactic reactions, development of inhibitors to factor VIII and potential dosing errors, the company should participate in EUHASS to collect long-term safety data.

2.8.2. Conclusions on the clinical safety

Overall, the Clinical Safety profile of AFSTYLA is comparable to other factor concentrates.

Experience of inhibitor formation in PUPs has been included as missing information and development of inhibitors has been included as an important identified risk.

Factor FVIII dosing errors might occur due to discrepancy of one-stage versus chromogenic test results. However, adverse reactions due to such dosing errors are not biological plausible and thus not anticipated. To avoid dosing errors a conversion factor of 2 has been introduced.

Hence, the CHMP considers the following measures necessary to address issues related to safety (please see RMP section below):

- In order to obtain further safety data (on the important identified and potential risks described in the RMP) in prophylaxis, on-demand long-term treatment and in surgery, the company should submit the results of Study 3001, a phase III open label, multicenter, extension study to assess the safety and efficacy of recombinant coagulation Factor VIII (rVIII-SingleChain, CSL627) in subjects with severe haemophilia A.

- In order to investigate the hypersensitivity/anaphylactic reactions, development of inhibitors to factor VIII and potential dosing errors, the company should participate in EUHASS to collect long-term safety data.

2.9. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Hypersensitivity and anaphylactic reactions Development of inhibitors
Important potential risks	Dosing errors based on assay (ChS vs OS) used for monitoring of FVIII levels Development of antibodies against CHO host cell proteins
Missing information	Experience of inhibitor formation in PUPs Experience in pregnancy and lactation, including labor and delivery Experience in geriatric patients (65 years and above)

Summary of safety concerns	
	Experience of use in patients for ITI (off-label use)

Pharmacovigilance plan

Activity / Study title (type of activity, study title [if known] category 1-3)*	Objectives	Safety concerns addressed	Status Planned, started,	Date for submission of interim or final reports (planned or actual)
Study 3001 A phase III open label, multicenter, extension study to assess the safety and efficacy of recombinant coagulation Factor VIII (rVIII-SingleChain, CSL627) in subjects with severe haemophilia A (category 3)	To obtain safety data in prophylaxis and on-demand long-term treatment, and in surgery. The primary objective of this study is to evaluate the safety of long-term use of rVIII-SingleChain.	All	Ongoing	Interim "snapshot" for 200 PTPs with 100 ED: Q2 2017 Projected submission for final CSR is Q4 2021.
Participation in EUHASS to collect long-term safety data (Category 3)	To review the available post-marketing data for safety concerns	Hypersensitivity/ anaphylactic reactions and development of inhibitors to factor VIII	Planned	Interim updates based on EUHASS reports will be included in each PSUR.

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC also considered that routine PhV is sufficient to monitor the effectiveness of the risk minimisation measures.

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
All	Prescription only medicine: rVIII-SingleChain is expected to be used under the supervision of a physician experienced in the treatment of haemophilia A.	None proposed.
Hypersensitivity and anaphylactic reactions	Sections 4.3 (Contraindications), 4.4 (Special warnings and precautions for se) and 4.8 (Undesirable effects) of the proposed SmPC includes appropriate information and advice.	None proposed.
Development of inhibitors	Section 4.4 (Special warnings and precautions for use) and 4.8 (Undesirable effects) of the proposed SmPC includes appropriate information and advice.	None proposed.
Dosing errors based on assay (ChS vs OS) used for monitoring of FVIII levels	Section 4.2 (Posology and method of administration) and 4.4 (Special warnings and precautions for use) of the proposed SmPC includes appropriate information and advice.	None proposed.
Development of antibodies against CHO host cell proteins	Sections 4.3 (Contraindications), 4.4 (Special warnings and precautions for use) and 4.8 (Undesirable effects) of the proposed SmPC includes appropriate information and advice.	None proposed.
Experience of inhibitor formation in PUPs	Section 4.2 (Posology and method of administration) of the proposed SmPC includes appropriate information and advice.	None proposed.
Experience in pregnancy and lactation, including labor and delivery	Section 4.6 (Fertility, pregnancy and lactation) of the proposed SmPC includes appropriate information and advice.	None proposed.
Experience in geriatric patients (65 years and above)	Section 4.2 (Posology and method of administration) of the proposed SmPC includes appropriate information and advice.	None proposed.
Experience of use in patients for ITI (off-label use)	FVIII concentrates are commonly used for ITI in patients who develop inhibitors to FVIII. Regimens and dosing of FVIII concentrates for this purpose are highly variable and dependent on local clinical practice.	None proposed.

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s).

Conclusion

The CHMP and PRAC considered that the risk management plan version 3.0 is acceptable.

2.10. Pharmacovigilance

Pharmacovigilance system

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

2.11. New Active Substance

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

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

2.12. Product information

2.12.1. User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the applicant and has been found acceptable for the following reasons:

The "QRD form for submission and assessment of user testing bridging proposals [EMA/355722/2014]" was used to provide adequate justification for bridging the Readability User Test results for Biostate 250 IU/600 IU to the proposed package leaflet of Afstyla. The bridging was justified with a comparison of both leaflets with regard to the key elements and identification of only minor differences between parent (Biostate) and daughter (Afstyla) leaflet. All key safety messages of the daughter PL are also present in the parent leaflet and the additional text is written in similar patient friendly language. The changes in wording of the daughter leaflets were considered to improve readability and the minor differences in the layout of the daughter PL were considered to maintain or improve clarity of the parent PL layout.

The justification is considered to be acceptable and no separate User Testing for Afstyla has to be provided.

2.12.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Afstyla (lonoctocog alfa) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

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

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Haemophilia A is a rare and serious, X-linked, recessive bleeding disorder that predominantly affects males and is characterized by a deficiency of FVIII. In patients with haemophilia A, the primary platelet-driven hemostasis is not affected, but generation of a stable, fibrin-rich clot is defective because inadequate amounts of thrombin are generated. Affected patients suffer from both spontaneous, non-traumatic bleeding episodes as well as substantially prolonged bleeding episodes upon injury. Rarely, life-threatening bleeding may also occur. Patients exhibit variable clinical phenotypes depending on the extent of residual activity (%) of the deficient FVIII that is used to classify the disease severity (WFH, 2012):

- <1% FVIII activity: severe haemophilia A
- 1% to 5% FVIII activity: moderate haemophilia A
- 5% to 40% FVIII activity: mild haemophilia A

Patients with severe haemophilia A bleed spontaneously into joints and muscles, which often results in permanent, disabling joint damage.

Afstyla is indicated for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA can be used for all age groups.

3.1.2. Available therapies and unmet medical need

Most patients with severe haemophilia require treatment of bleeding episodes and may require regular supplementation with intravenous (IV) factor replacement, either plasma-derived concentrate or recombinant. The goal of haemophilia therapy is to treat or prevent haemorrhage, thereby reducing disabling joint and tissue damage, and improving morbidity and the patient's quality of life. Replacement therapy with FVIII in haemophilia A provides a temporary correction of the factor deficiency and the bleeding tendencies. The prophylactic treatment course is variable and individually determined.

3.1.3. Main clinical studies

3.2. Favourable effects

Afstyla, a recombinant single-chain human coagulation factor VIII, was developed for replacement therapy of patients with haemophilia A.

Clinical efficacy of rFVIII-SingleChain has been investigated in two Phase 3 studies in adults and adolescents ≥ 12 years of age and in children <12 years of age in a step-wise approach.

rFVIII-SingleChain efficacy was investigated in treatment of bleeds, routine prophylaxis and perioperative management by assessment of treatment response rating, rFVIII-SingleChain consumption for a dose of 20-50 IU/kg administered 2 to 3 times weekly and determination of ABR. PK data serve as an important surrogate endpoint.

In the treatment of bleeds, response was assessed as "excellent" or "good" for more than 90% of rFVIII-SingleChain injections in both Phase 3 studies. More than 80% of the occurred bleeds were treated with one injection with a median dose of ~30 IU/kg in subjects ≥12 years and ~35 IU/kg in subjects <12 years of age. Median ABRs during prophylaxis are 1.14 in subjects ≥12 years and 3.69 bleeds/year in subjects <12 years of age.

A sufficient number of major surgeries were evaluated with haemostasis assessed as "excellent" or "good" in all of them.

Efficacy has been demonstrated by low ABR and FVIII-consumption comparable to marketed FVIII products and high-level of haemostatic efficacy in bleeding episodes and surgical setting.

3.3. Uncertainties and limitations about favourable effects

No important uncertainties about the key favourable effects have been identified.

3.4. Unfavourable effects

The risk-profile of Afstyla might be described by well-known risks from other Factor VIII concentrates together with anticipated risks according to specifically introduced changes of the molecule when compared with the native FVIII. AE-documentation from submitted Clinical studies 1001 (pivotal PK and efficacy-study), 3002 (paediatric study) and preliminary results from the ongoing extension study 3001 serves as the main source of information. Well-known risks include inhibitor development, hypersensitivity reactions, thrombo-embolic events, development of ADAs and anti-CHO antibodies.

For Afstyla, no de-novo inhibitor has been documented, so far. Hypersensitivity-reactions have been identified in nine subjects (rated as related in 6 subjects), of which one needed treatment with steroids and antihistamines for a limited period of time. There was one other patient with pre-existing inhibitor and positive ADAs who also experienced hypersensitivity.

Non-inhibitory ADAs were detected during the treatment among patients negative at baseline. No clinical relevance has been identified, yet.

3.5. Uncertainties and limitations about unfavourable effects

A high discrepancy was noted between results obtained with the OS and the ChS assay: the one-stage clotting assay result underestimates the factor VIII activity level compared to the chromogenic assay result by approximately 45%.. The linear relationship across critical FVIII activity levels demonstrated a consistent proportional decrease in OS FVIII activity measurements. Thus, the applicant proposes to include a conversion factor of 2 into the SmPC posology recommendations which should align OS activity levels with ChS activity results.

Factor VIII monitoring errors due to discrepancy of one-stage versus chromogenic-test-results might not have been identified under clinical trials conditions although potentially causing a top-up dose to be given, which, while unnecessary, would not result in an additional risk for the affected patient. In healthy individuals, FVIII circulates in plasma at concentrations of 50 to 150 IU/dL. However, FVIII is an acute-phase reactant, and plasma levels in healthy individuals without congenital bleeding disorders rise above 240% in acute situations such as surgery, sports, and infections. While chronically elevated FVIII levels have been identified as a risk factor for thrombosis in patients without congenital bleeding disorders, that is not the case for acutely elevated FVIII plasma activity. Given the short plasma half-life of FVIII in haemophilia patients, high doses (even in a prophylaxis regimen) would never reach steady state (ie, chronically elevated levels). Adverse reactions due to dosing errors caused by misinterpretation of monitoring results are not biologically plausible and therefore are not anticipated . This has been included an important potential risk.

Studies enrolled elderly patients only to a very low extent (two patients so far ≥60 years).

Hence, the CHMP considers the following measures necessary to address issues related to safety (please see RMP section):

- In order to obtain further safety data (on the important identified and potential risks described in the RMP) in prophylaxis, on-demand long-term treatment and in surgery, the company should submit the results of Study 3001, a phase III open label, multicenter, extension study to assess the safety and efficacy of recombinant coagulation Factor VIII (rVIII-SingleChain, CSL627) in subjects with severe haemophilia A.
- In order to investigate the hypersensitivity/anaphylactic reactions, development of inhibitors to factor VIII and potential dosing errors, the company should participate in EUHASS to collect long-term safety data.

3.6. Effects Table

Table 31: Effects Table for Afstyla- data cut-off: 29 May 2015

Effect	Short Description	Unit	rFVIII-SingleChain	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Pharmacokinetics						
Half-life, IR, Clearance	Standard PK parameters according to Clinical Guideline	diverse	~ 14 h (>12 years old) 1.7 -2.0 (>12 years old) ~ 3-4 ml/h/kg (<12y)	Comparator Advate in n=27 subjects	Consistent and reliable underestimation with One-stage assay / Requirements of Clinical Guideline met; Robustness evaluated; PK data in line with other rFVIII concentrates;	PK section
Prophylaxis						
ABR	Annualized bleeding rate (ABR)for 2 or 3 times weekly prophylaxis	Median number of bleeds/year	Study 1001: 1.14 (0;40.6) Study 3002: 3.69 (0;23.7) Age<6y 2.12 (0;17) Age 6 to <12y 5.11 (0;23.7)	none	ABR vary considerably between studies and depends on dosage; According to the Clinical Guideline, ABR has not been introduced as an efficacy parameter. Lack of definitions for a “bleed”, for “spontaneous” or “traumatic” nature of such bleed and individual evaluation factors are considered to be challenging when comparing numbers. ABR<2 comparable to marketed FVIII	Table 12 Table 23 Table 24

Effect	Short Description	Unit	rFVIII-SingleChain	Control	Uncertainties / Strength of evidence	References
					Higher ABR in the older age group	
Consumption per interval	Amount of administered FVIII	IU/kg per year	Study 1001: 4661.5 (2078;20789) Study 3002: 4109 (848;8601) Age <6y 4070 (848;8601) Age 6 to<12y 4147 (2142; 7875)	none	overall consumption across all regimens was similar between studies	Table 13 Table 25 Table 24
Haemostatic efficacy in bleeding episodes						
Haemostatic efficacy (Investigator's assessment)	4-point scale	%	Study 1001 Excellent: 71.1 Good: 21.2 Moderate: 6.1 Study 3002 Excellent: 85.3 Good: 11 Moderate: 3.5 Poor: 0.3	none	Subjective assessment results comparable to marketed FVIII respective case-narratives for moderate/poor response In major bleeds both median dose per injection (48.9 vs. 31.6) and median total dose (50.5 vs. 31.8) were higher than in study 1001. There was 1 major bleed in study 3002, whereas there were no major bleeds in study 1001. When comparing severe bleeds with mild or moderate bleeds, however, a higher consumption for severe bleeds could be seen, specifically in the paediatric study.	Table 10 Table 26
N/infusions per bleed	Number of injections required to achieve haemostasis	%	Study 1001 1 inf: 80.9 2 inf: 12.6 3 inf: 3.42 Study 3002 1 inf: 85.9 2 inf: 9.8 3 inf: 2.3			Table 11 Table 22

Effect	Short Description	Unit	rFVIII-SingleChain	Control	Uncertainties/ Strength of evidence	References
Perioperative haemostatic efficacy						
Haemostatic efficacy (Investigator's assessment)	4-point scale	%	Excellent:93,75 Good: 6.25	none	Subjective assessment results for major surgery comparable to marketed FVIII	Table 15
Unfavourable Effects						
Adverse Events of Special Interest						
Hypersensitivity	Clinically relevant symptoms of hypersensitivity	Case report	9 subjects (of which 6 subjects were considered to have related events) co-incidence with pre-existing inhibitor positive ADAs, positive re-challenge	n/a	Quality of queries might not be comparable with other factor-concentrates	Section 2.9 (clinical safety) Immunological Events

Abbreviations: BU Bethesda Units

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

A FVIII product's beneficial effects of maintaining haemostasis in haemophilia A patients are of obvious importance, since they result in an improvement of the quality of life and increased life-expectancy, and they could adequately be shown and expected for rFVIII-SingleChain.

Additional information in Previously Untreated Patients (PUPs) is crucial in the context of treatment in haemophilia A. Experience of inhibitor formation in PUPs has been included as missing information and development of inhibitors has been included as an important identified risk.
Hypersensitivities have been identified as relevant regarding number and quality. As current database for this newly developed single chain product is restricted, this entity of unfavourable effects requires further follow-up post-marketing. This will be monitored in Study 3001 and in EUHASS Registry (please see RMP).

3.7.2. Balance of benefits and risks

Based on the currently available data, the benefit-risk balance is considered to be positive.

3.7.3. Additional considerations on the benefit-risk balance

Not Applicable.

3.8. Conclusions

The overall B/R of Afstyla is positive.

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 Afstyla is favourable in the following indication:

Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA can be used for all age groups.
The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

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

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

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

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

Risk Management Plan (RMP)

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

An updated RMP should be submitted:

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

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

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that lonoctocog alfa is considered to

be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan EMEA-001215-PIP01-11-M04 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.