

Summary Basis for Regulatory Action

Date: May 19, 2016

From: Alexey Khrenov, PhD, Chair of the Review Committee

BLA/ STN: 125591/0

Applicant Name: CSL Behring Recombinant Facility AG

Date of Submission: 29 May 2015

PDUFA Goal Date: 28 May2016

Proprietary Name: AFSTYLA

Established Name: Antihemophilic Factor (Recombinant), Single Chain

Indication: In children and adults with hemophilia A (congenital Factor VIII deficiency) for: (1) on-demand treatment and control of bleeding episodes, (2) routine prophylaxis to reduce the frequency of bleeding episodes, and (3)perioperative management of bleeding .

Recommended Action: Approval

Signatory Authorities Action:

Offices Signatory Authority: Jay Epstein, MD, CBER/OBRR

I concur with the summary review.

I concur with the summary review and include a separate review to add further analysis.

I do not concur with the summary review and include a separate review.

Offices Signatory Authority: Mary Malarkey, CBER/OCBQ

I concur with the summary review.

I concur with the summary review and include a separate review to add further analysis.

I do not concur with the summary review and include a separate review.

Material Reviewed/ Consulted	Specific documentation used in developing the SBRA
Reviewer Name – Document(s)	Date
Clinical Review	Victor Baum
Clinical Pharmacology Review	Iftekar Mahmood
Statistical Review	Jiang Hu
CMC - Product	Alexey Khrenov, Natalya Ananyeva, Ze Peng
CMC - Facility	Donald Ertel

Lot Release Testing Plan In-support Testing	Karen Campbell, Claire Wernly Lokesh Bhattacharya, Hsiaoling Wang
Pharmacology/ Toxicology Review	Yolanda Branch
Bioresearch Monitoring Review	Christine Drabick, Haecin Chun
Labeling	Kristine Khuc
Establishment Inspection Report	Donald Ertel
Project Management	Thomas Maruna
Epidemiology	Adamma Mba-jonas

1. Introduction

STN 125591/0 is an original biologics license application (BLA) submitted by CSL Behring Recombinant Facility AG (CSLB) for Antihemophilic Factor (Recombinant), Single Chain with the proprietary name AFSTYLA. The active ingredient of AFSTYLA is a recombinant analogue of human Coagulation Factor (F) VIII with genetic mutations that removed most of the FVIII B-domain and 4 amino acids of the adjacent acidic a3 domain. The genetic manipulation, which will be described in detail below, also resulted in the expression of the protein as a single chain versus a heterodimer found in wild-type FVIII. The protein is expressed in a Chinese Hamster Ovary (CHO) cell line, and purified using traditional manufacturing methodologies. The product is supplied as a preservative-free, lyophilized formulation presented in 5 dosage strengths of 250, 500, 1000, 2000 and 3000 IU in single-use glass vials of 6 mL (250, 500 and 1000 IU) or 10 mL (2000 and 3000 IU) nominal capacity. AFSTYLA is reconstituted with sterile Water for Injection (sWFI) using a needleless transfer device, Mix2vial™, giving volumes of 2.5 mL (for 250, 500 and 1000 IU) and 5 mL (for 2000 and 3000 IU).

AFSTYLA is indicated to treat children and adults with hemophilia A (congenital FVIII deficiency) for: (1) on-demand treatment and control of bleeding episodes, (2) routine prophylaxis to reduce the frequency of bleeding episodes, and (3) perioperative management of bleeding.

STN 125591/0 was reviewed under the standard review schedule of the PDUFA V Program. CSLB submitted the BLA on 29 May 2015 and the PDUFA V action due date is 28 May 2016.

2. Background

AFSTYLA was developed for the U.S. market under IND 14791 for the control and prevention of bleeding episodes (BE), peri-operative management of bleeding, and routine prophylaxis to prevent or reduce the frequency of BE in children and adults with hemophilia A.

Hemophilia A affects 1 in 5,000 male births. The exact number of people living with hemophilia in the United States is not known, but currently the number is estimated to be about 20,000, of which 80% have hemophilia A. Several FVIII products are licensed in the United States for the treatment of people with hemophilia A.

The active ingredient in AFSTYLA is a recombinant single-chain analogue of Factor VIII (sc-rFVIII) produced in CHO cells. It is derived from a genetic construct in which the coding sequences for most of the FVIII B-domain and four amino acids of the adjacent acidic a3 domain were removed (amino acids 765 to 1652 of full-length FVIII). No new amino acids were added, but a new N-glycosylation site was formed by the resulting sequence at the junction of the heavy and light chain of FVIII. (b) (4)
the FVIII molecule in AFSTYLA is expressed as a single-chain. These changes were designed (b) (4)

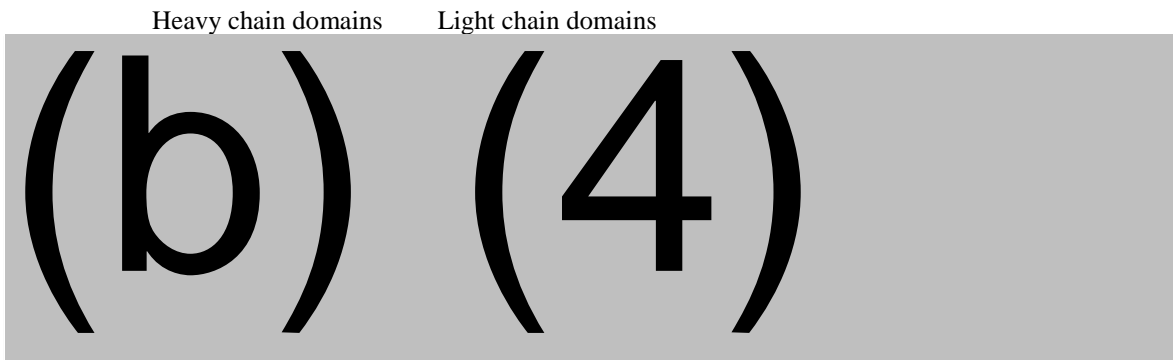
(b) (4)

of the single-chain FVIII molecule.

After activation by thrombin and removal of the (b) (4) B- and a3-domain, the activated rFVIII (rFVIIIa) molecule formed has an amino acid sequence identical to FVIIIa formed from endogenous, full-length FVIII. Therefore, AFSTYLA shares the same mechanism of action as other licensed FVIII products in hemostasis.

Figure 1 shows the structure of AFSTYLA; the domain structure, the linkage between the heavy and light chains of FVIII, the thrombin cleavage sites, and the N-glycosylation sites. The new glycosylation site is indicated by a glycan structure shown in red. The amino acid numbering is based on mature, full-length FVIII.

Figure 1: AFSTYLA structure.



AFSTYLA is labeled with FVIII activity determined by a chromogenic substrate (ChS) assay using a product-specific reference standard calibrated against the (b) (4) International Standard for FVIII Concentrate. Depending on the reference standard and reagents used in the assay, the FVIII activity in AFSTYLA can be approximately 2 times higher than that measured using a one-stage clotting (OS) assay. The cause of this difference is a (b) (4)

(b) (4). As the OS assay is more sensitive to the kinetics of the reaction than the ChS assay, this causes the discrepancy. However, there is no evidence to indicate that the (b) (4) of AFSTYLA (b) (4) affects its hemostatic function.

As a result of the assay discrepancy, under-estimation of FVIII activity in post-infusion patient plasma samples can be expected in the clinical settings because the OS assay using a plasma reference standard for FVIII activity is customarily used in the majority of clinical laboratories in the United States. To address this issue, CSLB has demonstrated that a correction factor of 2 can be used to determine FVIII activity levels with the OS assay. The use of this correction factor will be added to the prescribing information, and CSLB will implement a communication plan (see Section 9. Other Relevant Regulatory Issues) to educate healthcare

providers about the use and interpretation of laboratory assays to monitor patients treated with AFSTYLA.

3. Chemistry, Manufacturing and Controls (CMC)

a) Product Quality

Cell Bank System

The (b) (4) for B-domain deleted FVIII (deletion of amino acids 765-1652) with a linker containing an N-linked glycosylation site was engineered by (b) (4). (b) (4) for full-length human FVIII obtained from (b) (4). The CHO cell line expressing the sc-rFVIII protein was generated through (b) (4).

Master Cell Bank (MCB) and Working Cell Bank (WCB) were generated from this line.

(b) (4)

Characterization of the MCB, WCB (b) (4) was performed in accordance with the ICH Guideline Q5D: *Derivation and Characterization of Cell Substrates Used for Production of Biotechnological / Biological Products*, and included analyses for sterility, bacteriostasis / fungistasis, mycoplasma, cell line identity (by (b) (4)), and cell viability. Evaluation of the cell banks for safety with regard to adventitious viruses was performed in accordance with the ICH Guideline Q5A(R1): *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, and included relevant biochemical, biological (both in vitro and in vivo) and immunological tests. Genetic characterization was performed in accordance with the ICH Guideline Q5B: *Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products*. These studies confirmed (b) (4).

Together, the results of the characterization studies adequately qualify the MCB and WCB as the starting material for the manufacture of the drug substance for AFSTYLA and confirm the identity, safety and genetic stability of the cell substrate throughout the production process.

Manufacturing Process

The manufacture of AFSTYLA is divided into two main stages (see Figure 2) conducted at two manufacturing facilities. Production of the Bulk Drug Intermediate (BDI) takes place at

contract manufacturer (b) (4), which was not previously operating under a biologics license and was inspected during the review of this BLA. Production of the Bulk Drug Substance (BDS) and Final Drug Product (FDP) are performed at the FDA-licensed facility of CSLB's subsidiary CSLB Behring GmbH in Marburg, Germany.

Bulk Drug Intermediate and Bulk Drug Substance

(b) (4)

(b) (4)

Final Drug Product

(b) (4) used to manufacture (b) (4) batches of FDP. FDP batch size varies between approximately (b) (4) vials depending on which dosage presentation is manufactured. The FDP is provided as a lyophilized powder in single-use glass vials containing nominally 250, 500, 1000, 2000 and 3000 IU of FVIII activity (Table 1). The FDP is reconstituted with sWFI using a needleless device, Mix2vial.

Table 1: Nominal composition of reconstituted AFSTYLA

Ingredient	Nominal composition after reconstitution with sWFI					Function
	250 IU vial	500 IU vial	1000 IU vial	2000 IU vial	3000 IU vial	
Factor VIII activity	100 IU/mL	200 IU/mL	400 IU/mL	400 IU/mL	600 IU/mL	Active Substance
Sodium chloride	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Polysorbate 80	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Calcium chloride	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Sucrose	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
L-Histidine	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

Evaluation of Safety Regarding Adventitious Agents

For non-viral adventitious agents including bacteria, fungi, and mycoplasma, the potential of contamination of these agents is well controlled through the use of: (1) appropriate environmental control monitoring in the manufacturing process; (2) in-process controls, e.g., testing for bioburden and mycoplasma in (b) (4) steps including (b) (4). The potential of AFSTYLA to be contaminated with non-viral adventitious agents is further reduced by testing the FDP for sterility, endotoxins, and particulate matters. CSLB manufactures AFSTYLA according to cGMP regulations.

No human or animal derived raw materials are used in the manufacture and in the formulation of AFSTYLA. Thus, the potential risk of contamination by adventitious viruses or transmissible spongiform encephalopathy (TSE) agents is minimized.

The potential of contamination by infectious viruses in cell culture is well controlled in the manufacture of AFSTYLA, which is produced in a genetically modified CHO cell line. CSLB performed viral tests on the MCB for AFSTYLA consistent with ICH guideline Q5A(R1). All test results for endogenous and adventitious viruses were negative except for a positive result (b) (4) and the presence of (b) (4) MCB. The positive result of (b) (4) appears to be associated with the presence of (b) (4) that are considered to be non-pathogenic. Furthermore, all viral tests were negative except for (b) (4). CSLB routinely tests cell cultures used in the manufacturing process for adventitious viruses to ensure that viruses are below their detectable levels.

Additionally, the potential risk of viral contamination of AFSTYLA is further mitigated through two dedicated, orthogonal viral clearance steps: (b) (4)

CSLB has evaluated these viral clearance steps in relevant down-scale studies using model viruses. The viruses selected for the studies include (b) (4)

The wide range of physico-chemical properties of these model viruses demonstrates the ability of the manufacturing process to reduce potential viral contamination from AFSTYLA. Down-scale studies on the relevant steps resulted in the following overall log reduction factors, in parenthesis, for these viruses: (b) (4)

These results support the proposal that viral clearance is effective in the manufacture of AFSTYLA.

Process Validation and Qualification

Process Performance Qualification (PPQ) was accomplished in three separate parts, corresponding to the three major stages of production, BDI^{(b) (4)} PPQ batches from (b) (4) runs), BDS^{(b) (4)} PPQ batches) and FDP^{(b) (4)} PPQ batches). Although the PPQ batches for the BDI process and PPQ batches for the BDS process were performed in separate and independent PPQ campaigns, the corresponding non-PPQ portions of the processes were performed under representative commercial process conditions. PPQ for FDP consisted of the manufacture of consecutive batches covering each of the filling sizes. The PPQ data demonstrated that the manufacturing processes for AFSTYLA BDI, BDS and FDP were successfully qualified.

In addition to the PPQ studies, several ancillary validation studies were performed to support the consistency of the manufacture of AFSTYLA BDI and BDS. The studies included *In-Process Hold Time Validation*, (b) (4) *Validation*, *Mixing Validation*. For AFSTYLA FDP, results of several validation studies were provided as well, including *Validation of filling*, *Validation of lyophilization cycles*, *Validation of (b) (4) of the drug substance*, *Validation of mixing steps*, *Validation of hold times*, *Validation of (b) (4)*

CSLB developed Continued Process Verification (CPV) plans at both (b) (4) and CSLB Behring GmbH to ensure the validated state of the AFSTYLA manufacturing process throughout the product lifecycle. The CPV program is designed to collect process data and perform statistical evaluation of the dataset in order to routinely confirm the validated state and to identify and evaluate planned and unplanned changes in the manufacturing process.

In-Process Controls

The process control strategy was developed using a risk-based and science-based approach based on regulatory guidance provided by ICH Q8 – Q10 that ensures the consistency of the manufacturing process and product quality.

Potency

The potency of AFSTYLA is expressed in international units of FVIII activity and determined using an in vitro ChS assay. Comparison of the potency assignments for AFSTYLA using the ChS and OS assays revealed an approximate 2-fold difference; with the OS assay giving a lower value than the ChS assay. CSLB conducted non-clinical and in vivo investigation of the hemostatic effects of AFSTYLA and concluded from the data that a potency assignment using the ChS assay results in the most accurate assignment of 1 IU to an amount of protein that matches the hemostatic potential of FVIII in 1 mL of plasma in healthy individuals. Consequently, the material used in all the clinical studies received a potency assignment based on the ChS assay.

To support the selection of a potency assay for AFSTYLA, several in vitro investigations were performed to examine the different aspects of FVIII potency testing and related functional characterizations of AFSTYLA:

(b) (4)

(b) (4) [Redacted text block]

(b) (4) [Redacted text block]

(b) (4) [Redacted text block]. As a result, the potency assignment by the OS assay would result in a significantly higher amount of FVIII protein in the vial and dose, whereas the ChS-based potency allows for a protein content to be comparable to that of other recombinant B domain-deleted FVIII products.

There is no evidence to indicate that the (b) (4) of AFSTYLA (b) (4) affects its hemostatic function. All the materials used in the clinical studies received a potency assignment based on the ChS assay. The efficacy was demonstrated for all the proposed indications and no evidence of under-dosing was observed.

As a result of the assay discrepancy, under-estimations of FVIII activity in post-infusion plasma samples can be expected in clinical settings because the OS assay with a plasma reference standard for FVIII activity is customarily used in the majority of the clinical

laboratories in the United States. This underestimation may potentially lead to patients receiving more AFSTYLA than is needed.

CSLB central laboratory characterized the relationship between the OS and ChS assays for AFSTYLA in the pharmacokinetics (PK) investigations of 130 subjects from the two clinical trials used to support licensure. From these data, CSLB claims a strong linear relationship between the ChS and OS assay results, with the OS assay results consistently being approximately 50% lower than the ChS assay results. Therefore, CSLB claims that the AFSTYLA FVIII activity data obtained using the OS assay can be aligned with that obtained using the ChS assay by multiplying the OS assay result by a correction factor of two. These results were confirmed in a field study involving (b) (4) clinical laboratories (including 13 from the United States). FDA agreed with CSLB's proposal to include the conversion factor, but noted that specific measures are required to adequately convey this information to clinicians, the hemophilia community, and to all those involved in the care of patients with hemophilia A in order to facilitate adequate monitoring, and to prevent over-dosing of AFSTYLA. To address this issue, specific communication and labeling strategies were developed by CSLB. These are discussed below.

A single primary product-specific potency standard calibrated against the (b) (4) WHO International Standard for Factor VIII Concentrate was developed from a (b) (4) batch of AFSTYLA manufactured at commercial scale and is currently used as a reference standard for all in-process and release testing of AFSTYLA. The structural and functional properties of this reference standard are extensively characterized. Prior to the development of this product-specific reference standard, the (b) (4) WHO International Standard for Factor VIII Concentrate and (b) (4) product standard (also calibrated against the (b) (4) WHO International Standard for Factor VIII Concentrate) were used for potency assignment.

Elucidation of Structure and Other Characteristics

The structure and function of AFSTYLA were characterized in a series of studies, which also examined the comparability of AFSTYLA batches manufactured at different sites and scales of the manufacturing process during product development. Functional characterization indicated similarity between AFSTYLA and licensed plasma-derived and rFVIII products in several parameters tested, except for the significant discrepancy between results from the OS and ChS assays described above.

Minimal BDS lot-to-lot variability was observed between AFSTYLA batches produced at different scales or process iterations.

Release specifications

The specifications of BDI, BDS and FDP are summarized in Tables 2-4 below. The methods and established specifications are based on manufacturing experience and available safety and efficacy data.

(b) (4)

Table 4: AFSTYLA FDP Specifications

Test	Parameter Monitored	Specification
Practicability and organoleptic properties	Quality	Lyophilized powder: White to slightly yellow powder (b) (4) Dissolution time: max. (b) (4) Reconstituted solution: Almost colorless, clear to slightly opalescent solution
(b) (4)	Quality	(b) (4)
(b) (4)	Quality	(b) (4)
Residual moisture	Quality	(b) (4)

Test	Parameter Monitored	Specification
Sodium	Quality	(b) (4)
Calcium	Quality	(b) (4)
Sucrose	Quality	(b) (4)
Histidine	Quality	(b) (4)
Polysorbate 80	Quality	(b) (4)
(b) (4)	Purity	(b) (4)
Protein composition (b) (4)	Purity	(b) (4)
ChS FVIII activity	Potency Identity	(b) (4)
Protein concentration	Quality	(b) (4)
(b) (4) FVIII activity	Purity	(b) (4)
Sterility	Purity	(b) (4) 21 CFR, (b) (4)
Bacterial endotoxins	Purity	(b) (4)
Particulate matter	Purity	(b) (4)
(b) (4)	Quality	(b) (4)

Analytical Methods

The release methods were validated for their suitability for the intended use. The respective reference standards and their maintenance program were established. The results of in-support testing for potency and purity of the FDP were within the proposed specifications. Method qualifications for: 1) bioburden; 2) endotoxins; and 3) sterility tests were qualified in

accordance with (b) (4) respectively, by demonstrating that the AFSTYLA matrix is suitable for these intended test methods.

Facilities Review/Inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facilities involved in the manufacture of FSTYLA are listed in the table below. The activities performed and inspectional histories are noted in the table and are further described in the paragraphs that follow.

Table 5. Manufacturing Facilities for AFSTYLA

Name/Address	FEI number	DUNS number	Inspection/waiver	Justification/Results
<i>Drug Substance</i> Manufacturing and Testing (b) (4)	(b) (4)	(b) (4)	Pre-License Inspection	(b) (4) NAI
<i>Drug Substance</i> Manufacturing and Testing <i>Drug Product</i> Formulation, Fill/Finish, Labeling & Packaging, Testing CSL Behring GmbH (CSLB) Emil-von-Behring-Strasse 76 D-35041 Marburg Germany	3003098680	326530474	Waived	CBER DMPQ May 28- June 5, 2015 VAI

CBER conducted a pre-license inspection (PLI) of (b) (4), drug substance manufacturing and testing facility, from (b) (4). At the end of the inspection, no Form FDA 483 was issued. The inspection was classified as no action indicated (NAI).

CBER DMPQ performed a pre-license inspection in support of another product of CSLB from May 28 to June 5, 2015. The inspection was classified as voluntary action indicated (VAI), and the corrective actions were reviewed by CBER and found to be adequate.

Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product will not alter significantly the concentration and distribution of naturally occurring substances and no extraordinary circumstances exist that would require an environmental assessment.

Container/ Closure

The drug product is filled into 6 & 10 mL (b) (4) glass vials (b) (4) closed with bromobutyl rubber stoppers (b) (4) (b) (4). The stoppers are secured by combination caps consisting of an aluminum crimp cap with a concentric hole and an integrated polypropylene plastic disc. CSLB performed the container closure integrity testing at the Marburg, Germany facility, employing a (b) (4) test method; all acceptance criteria were met.

Stability

The stability data support that the BDS can be stored at (b) (4). FDP can be stored for 3 years at +5 °C including single period of up to 3 months at +25 °C if within the expiration date. If the product is removed from refrigeration and stored at room temperature, the product insert instructs the patient to record on the top flap of the carton the date that AFSTYLA is removed from refrigeration. After storage at room temperature, AFSTYLA should not be returned to the refrigerator. The product then expires after storage at room temperature for 3 months or after the expiration date on the product vial, whichever is earlier. The reconstituted product (after mixing dry product with diluent) can be stored at 2°C to 8°C or at room temperature for up to 4 hours.

Exemption from CBER Lot Release

Under the provision described in Federal Register (FR) 58:38771-38773 and the 60 FR 63048-63049 publication (December 8, 1995), routine lot-by-lot CBER release is not required for AFSTYLA because it is a well-characterized recombinant product. Thus, exemption of AFSTYLA from CBER Lot Release is justified. CBER has performed in-support testing of commercial scale AFSTYLA product lots of 250 IU, 500 IU, 1000 IU and 3000 IU nominal potencies. Test results were deemed mostly consistent with the proposed commercial release specifications, except for (b) (4) (see below).

SIGNIFICANT ISSUES RESOLVED DURING THE BLA REVIEW

The following substantive issues were resolved during the review of the AFSTYLA BLA:

a. Deficiencies in specifications

Multiple deficiencies were found in the specifications of the BDS and FDP. The quantitative acceptance criteria in the original application were established arbitrarily. The justifications of specifications were not supported by statistical analysis of manufacturing data, so the specifications did not allow for adequate control of the manufacturing process. CSLB successfully addressed these concerns by re-assessing the manufacturing data, revising, and justifying the specifications. Since limited number of (b) (4) batches were manufactured to date, CSLB also committed to establish the acceptance criteria for some parameters when more data are available from the commercial manufacturing process for statistical analysis.

Additionally, the parameters used to establish the acceptance criteria for the control of (b) (4) were found to be insufficient for their intended purposes. These specifications were revised by establishing acceptance criteria based on the analysis of parameters which were not previously tested.

b. Deficiencies in method validations

Review of method validation found multiple deficiencies, including incomplete validation of some parameters, validation of incorrect assay ranges, and acceptance criteria inappropriate for assessing method performance. CSLB satisfactorily addressed these review concerns by conducting additional validation studies, and preparing new SOPs and test instructions. These newly developed validation data were used to confirm the validity of the data associated with process development, qualification and verification, and comparability studies.

c. Inconsistency between batch analysis and in-support testing for (b) (4) .

During in-support testing of 250 IU batches (b) (4)

. As testing instructions provided in the BLA specifies (b) (4), this result was inconsistent with CSLB’s batch analysis data for the lots analyzed, which were all below the specification limit (b) (4). Per FDA request, CSLB provided an explanation regarding the nature of (b) (4)

. As requested by FDA, CSLB performed further studies and modified the test instruction to more clearly define (b) (4)

as well as information provided by CSLB, this finding is not affecting the safety of the product or reflecting any problems with manufacturing consistency. CSLB committed to improve and validate (b) (4)

d. Deficiencies in post-approval stability protocols

The original BLA lacked detailed post-approval stability protocols for DP and cell banks. As requested by the FDA, CSLB submitted modified stability protocol for DP, including tests and test time-points, and stability program for the MCB and WCB, which includes the assessment of cell culture growth parameters and analytical (b) (4) from actual manufacturing or dedicated pilot-scale runs at specified intervals. Per FDA request, the MCB and WCB protocol was further revised to include genetic stability testing – the verification of (b) (4) – and is adequate.

Conclusion

The CMC data support the quality and safety of AFSTYLA to be used in the treatment of children and adults with hemophilia B.

4. Nonclinical Pharmacology/Toxicology

General Considerations

The safety and effectiveness of AFSTYLA were characterized in a nonclinical program that included in vivo proof-of-concept testing in a murine model of hemophilia A, as well as in vivo PK, local tolerability, and single and repeat-dose toxicity studies in FVIII replete (i.e., wild-type) rats, and (b) (4) monkeys. A risk assessment of the potential extractable and leachable components present in the AFSTYLA (b) (4), as per the ISO 10993 standards and using clinical experience, was also completed.

Previous experience with similar recombinant and plasma-derived FVIII products has demonstrated that the toxicities of exogenously administered FVIII in treated animals and patients are extensions of its pharmacologic activity, i.e. hypercoagulability of blood, thrombosis, and thrombo-embolus formation. Additional expected nonclinical findings are development of neutralizing and non-neutralizing antibodies directed against the human FVIII protein (i.e. immunogenicity), with the potential to cross-react and neutralize endogenous FVIII in wild-type animals and potential increase in inhibitor antibody titer levels in patients with hemophilia A.

Nonclinical Findings

Pharmacology

Pharmacology (proof-of-concept) studies were conducted in a murine model of hemophilia A (i.e. mice with a naturally occurring mutation/deletion of FVIII function). Hemophilic mice were dosed intravenously with increasing doses of AFSTYLA, or another approved human rFVIII product. AFSTYLA treatment of hemophilic mice with doses approximately equivalent to two times the human starting dose decreased blood loss, time to hemostasis and activated partial thromboplastin times (aPTT) to within normal limits. Under the conditions tested, AFSTYLA was as effective as the approved FVIII product, based on total blood loss at comparable doses. There were no effects of AFSTYLA or the other approved FVIII preparations on the hematology profiles in mice as compared to prior to dosing (i.e. baseline), and no serious adverse effects or evidence of thrombogenicity were reported.

In summary, animal studies with AFSTYLA showed the expected pharmacologic i.e. pro-coagulant activity in a murine model of hemophilia A, and the results were similar to those obtained with other approved human FVIII products. There was no evidence of thrombosis or any other serious adverse effects. The data from these pharmacology studies were used as proof-of-concept to support the initiation of clinical trials, and are reflected in the pharmacology section of the AFSTYLA package insert.

Pharmacokinetics

PK studies with AFSTYLA were conducted in (b) (4) monkeys and rats at doses up to 2-fold the clinical starting dose. The PK profile of AFSTYLA in (b) (4) monkeys showed a dose-dependent increase in the PK parameters measured (C_{max}, and AUC₂₄), a similar trend was seen with the approved FVIII comparator products. In rats administered up to 2-fold the clinical starting dose of AFSTYLA, the PK profile was similar to three other licensed FVIII products.

Toxicology

Nonclinical toxicity studies conducted with AFSTYLA in rats and (b) (4) monkeys did not identify any unexpected findings or significant safety concerns. There were no reported systemic or tissue pathologies in FVIII-replete monkeys and rats dosed with a single, intravenous injection of AFSTYLA at doses up to 30-fold greater than the clinical starting dose. In a repeat-dose toxicity study, (b) (4) rats were injected daily for 28 days with AFSTYLA at doses of up to 25-fold greater than the clinical starting dose. Statistically significant differences in activated prothrombin time were reported for the highest dose group; however, this finding was not consistent either between sexes or between different AFSTYLA dose groups. A 28-day, repeat dose toxicity study with AFSTYLA was conducted in (b) (4) monkeys; groups of animals were dosed every day for 28 days by bolus intravenous injection with AFSTYLA at doses equal to, and up to 25-fold greater than the clinical starting dose. Based on the results of this study, AFSTYLA was well tolerated, with no findings indicative of systemic toxicity, and no reported pro-thrombogenic properties or adverse local tolerance. A local tolerance study conducted in rabbits administered the clinical dose of AFSTYLA either by intravenous, paravenous or intra-arterial injection revealed acceptable levels of inflammation and edema at the injection sites.

Special Toxicology Studies

No animal carcinogenicity, in vivo mutagenicity, fertility, reproductive toxicity or teratogenicity studies were conducted with AFSTYLA. AFSTYLA is a recombinant, human protein; animals receiving repeated doses of the product developed antibodies against FVIII that both accelerated clearance of the protein and in some cases, neutralized its pro-coagulant activity. Therefore, long-term, repeat-dose toxicity studies, as well as the standard carcinogenicity bioassay (i.e. two years of daily AFSTYLA dosing in both rats and mice) were not feasible to conduct.

The standard battery of genotoxicity testing as recommended in the International Conference on Harmonization (ICH) S2 guidance documents was not conducted with AFSTYLA as per the ICH S6 guidance on biotechnology-derived protein therapeutics these studies are not required. The lack of carcinogenicity, mutagenicity and chronic toxicity data are addressed in the appropriate section(s) of the AFSTYLA package insert.

No nonclinical reproductive or developmental toxicity studies with AFSTYLA were conducted in support of this application. The package insert includes a statement that nonclinical reproductive and developmental toxicity studies with AFSTYLA have not been conducted, and the product should be used in pregnancy only if clearly needed. This labeling is consistent with that included in prescribing information for other approved recombinant human coagulation factors for the treatment of hemophilia A or B.

Toxicological Risk Assessment Analysis

A toxicological risk assessment analysis was also provided in this submission, and provided identification and safety qualification of the extractable and potential leachable substances from the components used in the AFSTYLA manufacturing process. The results of this risk analysis indicated that the levels of potential leachable or extractable impurities were

acceptable, as they were significantly lower than the maximally allowed daily exposure levels identified from extensive clinical and nonclinical experience. The risk of toxicity from the presence of these compounds at the levels identified to patients with hemophilia A receiving intravenous doses of AFSTYLA was considered minimal, and acceptable considering the benefit of FVIII replacement therapy in this patient population.

Conclusion

In conclusion, the data from the nonclinical program with AFSTYLA suggest that its safety profile supports its use for the proposed indications of on-demand treatment and control of bleeding episodes, routine prophylaxis to reduce the frequency of bleeding episodes, and perioperative management of bleeding in adults and children with hemophilia A.

5. Clinical Pharmacology

Study Title: CSL627_1001 “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 Hemophilia A, and a repeat PK, safety and efficacy study”

This was an open-label, international, multicenter, cross-over study and consisted of three parts.

Part 1: There were 27 subjects 18-65 years of age, who had been diagnosed with severe hemophilia A with <1% FVIII:C levels. This part of the study included a single-sequence crossover PK comparison of Advate and AFSTYLA. Subjects received a single intravenous (IV) dose of 50 IU/kg Advate followed by the same dose of AFSTYLA after a 4-day wash-out period. Blood samples for PK study were taken through 72 hours. All PK samples were analyzed by the ChS assay and OS assay. PK parameters calculated from the ChS assay only were analyzed to assess the comparative bioavailability of AFSTYLA and Advate after dose-adjustment.

Part 2: This part assessed efficacy, safety, and PK of AFSTYLA with continued dosing from Part 1. The first five subjects received on-demand treatment to confirm the hemostatic potential of AFSTYLA, while the remaining subjects received either on-demand or prophylaxis treatment based on their preference and investigator discretion.

Part 3: This part assessed the safety and efficacy of AFSTYLA with continued dosing of new subjects, and included a repeat PK assessment for at least 13 subjects. All subjects enrolled from Japanese sites participated in the PK analysis. After PK assessment, subjects then began on-demand or prophylaxis treatment for at least 50 Exposure Days (ED).

The results of the study are summarized as follows:

- After a single IV dose of 50 IU/kg, the mean clearance and half-life of AFSTYLA by ChS assay were 2.55 ± 0.74 mL/hour per kg and 14.7 ± 3.7 hours, respectively. The

mean clearance and half-life of AFSTYLA by OS assay were 4.13 ± 1.31 mL/hour per kg and 15.5 ± 4.9 hours, respectively. Although the half-life of AFSTYLA was not different between ChS and OS assays, the clearance was 1.6-fold higher by the OS assay than by the ChS assay. C_{max} , $AUC_{(0-\infty)}$, and IR values by the OS assay were 49%, 63%, and 48% of the values determined by the ChS assay.

- The 90% confidence interval (90% CI) for C_{max} ranged from 90.5 to 104.6, indicating that C_{max} met the bioequivalence criteria. The 90% CI interval for $AUC_{(0-\infty)}$ failed to meet the bioequivalence criteria (range = 117.8-159.9).
- The PK of single and repeat dose (3 to 6 months after the initial PK) of AFSTYLA was comparable.
- Although the half-life of AFSTYLA in subjects ≥ 12 to < 18 years of age was comparable to those in subjects ≥ 18 years, clearance (based on per kg body weight) was 31% and 24 % higher in subjects ≥ 12 to < 18 years of age than subjects ≥ 18 years of age by ChS and OS assays, respectively.
- The PK parameters were comparable between non-Japanese and Japanese subjects.

Study Title: CSL627_3002 “A Phase III open-label pharmacokinetic, efficacy and safety study of rVIII-Single Chain in a pediatric population with severe Hemophilia A”

The PK study of FVIII was conducted in children < 6 years ($n = 20$) and 6 to < 12 years ($n = 19$) of age. The children received 50 IU/kg FVIII dose. Blood samples were taken at times 0, 1, 5, 10, 24, and 48 hours post-dose. The FVIII activity was measured by both ChS and OS assays.

In children < 6 years, by ChS the in-vivo recovery (IVR), half-life and clearance were 1.61 (IU/dL)/(IU/kg) (%CV = 21.4), 10.5 hours (%CV = 28.6), and 5 mL/hour per kg (%CV = 30.3), respectively. In this same age group, by OS assay the in vivo recovery, half-life and clearance were 0.9 (IU/dL)/(IU/kg) (%CV = 82.8), 11.1 hours (%CV = 48.8), and 9.73 mL/hour per kg (%CV = 45.4), respectively.

In children 6 to < 12 years of age, by ChS assay the in vivo recovery, half-life and clearance were 1.66 (IU/dL)/(IU/kg) (%CV = 19.7), 10.2 hours (%CV = 19.4), and 4.63 mL/hour per kg (%CV = 29.5), respectively. In this same age group, by OS assay the in vivo recovery, half-life and clearance were 0.84 (IU/dL)/(IU/kg) (%CV = 20.3), 10.3 hours (%CV = 26.6), and 8.44 mL/hour per kg (%CV = 42.4), respectively.

The results of the study indicate that the aforementioned PK parameters were comparable between children < 6 years and 6 to < 12 years of age by both assay methods (comparison within the assay method). The ChS assay provided lower clearance and higher IVR than the OS assay in both age groups. However, the half-life of FVIII was comparable by both assay methods between the two age groups. The OS assay method produced higher %CV than ChS assay in children < 6 years of age.

Comparison of clearance and half-life across age groups

Chromogenic Substrate Assay

The clearance and half-life of FVIII in adults (>18 years) were 2.55 mL/hr per kg and 14.7 hours, respectively. In children <6 years, clearance and half-life were 5 mL/hour per kg and 10.5 hours, respectively. The clearance of FVIII in this age group was approximately 2-fold higher and half-life was approximately 4 hours shorter than adults.

In children 6 to <12 years of age, clearance and half-life were 4.6 mL/hour per kg and 10.2 hours, respectively. The clearance of FVIII in this age group was approximately 1.8-fold higher and half-life was approximately 4 hours shorter than adults.

One-stage Clotting Assay

The clearance and half-life of FVIII in adults (>18 years) were 4.1 mL/hr per kg and 15.5 hours, respectively. In children <6 years, clearance and half-life were 9.7 mL/hour per kg and 11.1 hours, respectively. The clearance of FVIII in this age group was approximately 2.4-fold higher and half-life was approximately 4 hours shorter than adults.

In children 6 to <12 years, clearance and half-life were 8.4 mL/hour per kg and 10.3 hours, respectively. The clearance of FVIII in this age group was approximately 2-fold higher and half-life was approximately 5 hours shorter than adults.

Conclusions

- The half-life of AFSTYLA is approximately 15 hours in adults and approximately 10 hours in children under 12 years of age.
- The PK of single and repeat dose (3 to 6 months after the initial PK) of AFSTYLA is comparable.
- The PK parameters were comparable between non-Japanese and Japanese subjects.
- The clearance (body weight adjusted) of FVIII is almost 2-fold higher in children <12 years of age compared with older males. Dose adjustment is needed in this age group.

6. Clinical/ Statistical

a) Clinical Program

Summary of Clinical Trials

Clinical trials for AFSTYLA were conducted under IND 14791. Data from two clinical trials (CSL627_1001 and CSL627_3002) were submitted to support the safety and efficacy of AFSTYLA for the proposed indications of: (1) on-demand treatment and control of BE, (2) routine prophylaxis to reduce the frequency of BE, and (3) perioperative management of bleeding in adults and children with hemophilia A (congenital Factor VIII deficiency). Both trials evaluated the PK, safety and efficacy of AFSTYLA.

The clinical trials submitted to support the safety and efficacy of AFSTYLA are summarized in Tables 6A and 6B below.

Table 6A: Clinical Trial Including Adults and Adolescents

Trial CSL627_1001			
Trial Design	Open-label, international, multicenter, cross-over, efficacy, safety, and PK trial consisting of 3 parts plus a surgical sub-study		
	Part 1	Single-sequence crossover PK comparison Advate and AFSTYLA	
	Part 2	<ul style="list-style-type: none"> • Efficacy and safety AFSTYLA • Continued dosing from Part 1 • On-demand or prophylaxis treatment 	
	Part 3	<ul style="list-style-type: none"> • Efficacy and safety AFSTYLA • Continued dosing of new subjects • Repeat PK assessment of at least 13 new subjects • On-demand or prophylaxis treatment 	
Primary Objectives	<ul style="list-style-type: none"> • Characterize PK profile • Demonstrate efficacy in prevention and treatment of BE • Demonstrate efficacy of routine prophylaxis treatment over on-demand treatment • Characterize rate of inhibitor formation • Demonstrate efficacy in surgical prophylaxis 		
Subjects	Main Inclusion Criteria	<i>Parts 1 and 2</i>	Adult males \geq 18 years
		<i>Part 3</i>	Males \geq 12 to 65
		<i>Parts 1, 2, and 3</i>	<ul style="list-style-type: none"> • Severe hemophilia A (FVIII:C<1%) • > 150 prior ED with a FVIII
	Main Exclusion Criteria	<ul style="list-style-type: none"> • Any history of or current FVIII inhibitors or any first order family history of FVIII inhibitors • Any known hypersensitivity (allergic reaction or anaphylaxis) to any FVIII product or hamster protein • Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months prior to Day 1 • Currently receiving intravenous immunomodulating agents such as 	

		immunoglobulin or chronic systemic corticosteroid treatment		
	Disposition	175 enrolled; 174 treated (14 subjects \geq 12 to < 18 years) ; 161 completed* <ul style="list-style-type: none"> • 91 PK (Parts 1 and 3) • 173 prophylaxis and on-demand** <ul style="list-style-type: none"> ○ 146 prophylaxis ○ 27 on-demand ○ 13 surgical sub-study 		
Regimens	Part 1	50 IU/kg AFSTYLA or Advate single sequence crossover PK with final blood draw up to 72 h post-infusion		
	Part 2	<ul style="list-style-type: none"> • First 5 subjects from Part 1 received on-demand treatment to confirm AFSTYLA's hemostatic potential • Remaining subjects received either on-demand or prophylaxis treatment 		
		<i>Prophylaxis</i>	20-40 IU/kg every 2 days or 20-50 IU/kg 2 to 3 times/week	
		<i>On-demand</i>	<ul style="list-style-type: none"> • Dose similar to the FVIII product used before enrollment for same type BE • Median dose/infusion/BE 31.7 (range: 6 to 84) IU/kg 	
	Part 3	PK as in Part 1 and treatment as in Part 2		
Surgical sub-study	<ul style="list-style-type: none"> • Minimum of 5 subjects from either Parts 2 or 3 • Dose regimen individualized for type of surgery and subject's clinical status 			
Treatment Duration	Part 1	Single dose – PK samples at pre-dose, 0.5, 1, 4, 8, 10, 24, 28, 32, 48 and 72 h		
	Part 2	Subjects continue in the study until 50 ED and 6 months of treatment, or until at least 104 subjects reached 50 ED		
	Part 3	Subjects continue in the study until 50 ED and 6 months of treatment, or until at least 104 subjects reached 50 ED		
	Surgical sub-study	13 subjects underwent 16 surgeries		

*8 subjects withdrew consent; 1 discontinued based on the physician's decision; 1 had knee surgery; 1 had 55 ED but did not reach 6 months; 2 did not reach 50 ED

**1 subject completed PK assessment but withdrew consent before entering treatment regimen

Table 6B: Pediatric Clinical Trial

Trial CSL627_3002			
Trial Design	International, multicenter, open-label, efficacy, safety, and PK trial in pediatric subjects with severe hemophilia A <ul style="list-style-type: none"> Optional single-dose PK period followed by treatment period 		
Primary Objectives	Evaluate efficacy in treatment of major and minor BEs		
Subjects	Main Inclusion Criteria	<ul style="list-style-type: none"> Males <12 years Severe hemophilia A (FVIII:C<1%) > 50 prior ED with FVIII products 	
	Main Exclusion Criteria	<ul style="list-style-type: none"> Any history of, or current, FVIII inhibitors or any first order family history of FVIII inhibitors Any known hypersensitivity (allergic reaction or anaphylaxis) to any FVIII product or hamster protein Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months prior to Day 1 Currently receiving intravenous immunomodulating agents such as immunoglobulin or chronic systemic corticosteroid treatment 	
	Disposition	84 enrolled; 84 treated; 82 completed*** <ul style="list-style-type: none"> 35 subjects < 6 years <ul style="list-style-type: none"> 20 PK 0 on-demand 35 prophylaxis 49 subjects 6 to < 12 years <ul style="list-style-type: none"> 19 PK 3 on-demand 46 prophylaxis 	
Regimens	PK	Single IV dose of 50 IU/kg with final blood draw 48 h post-infusion	
	Treatment	<i>Prophylaxis</i>	<ul style="list-style-type: none"> Dosing 15 to 50 IU/kg every 2 days; or 2 to 3 times/week ; or Dose and frequency determined by investigator based on historical FVIII dosing and available PK data
		<i>On-demand</i>	<ul style="list-style-type: none"> Dosing per investigator discretion Median dose/infusion /BE was 27.3 (range: 16 to 76)

		IU/kg
Treatment	PK	Single dose – PK samples at pre-dose, then at 1 h, 5 h, 10 h, 24 h, and 48 h
Duration	Treatment	<ul style="list-style-type: none"> • Treatment continued until 25 subjects in each age group (< 6 years and ≥ 6 to < 12 years) achieved 50 EDs • Median time on study was 5.6 months (range: 1 to 11 months)

***1 subject withdrew for treatment-emergent AE (TEAE) of arthralgia; 1 withdrew for a series of complex social circumstances, including suspected Munchhausen by proxy

Demographics of the study population

A total of 258 subjects were exposed to AFSTYLA in clinical trials CSLB627_1001 and CSLB627_3002. Subjects in the pooled results of the two clinical trials had the following characteristics:

- Age
 - 18 to 65 years: 160
 - 12 to <18 years: 14
 - 6 to <12 years: 49
 - <6 years: 35
- Sex: All males
- Race
 - Caucasian: 187
 - Asian: 53
 - Black/African American: 14
 - Other: 4¹
- Ethnicity
 - Hispanic/Latino: 14
 - Not Hispanic/Latino: 242
 - Not Reported: 2

Disposition of study subjects

Sixteen subjects were discontinued from the trials after treatment, including two pediatric subjects who were discontinued as a result of adverse events (AEs).

In Trial CSLB627_1001:

- No subjects withdrew due to an AE.
- Of the 13 subjects who withdrew, 8 withdrew consent, 1 withdrew based on a physician decision and 4 withdrew for “other” reasons (**Subject 040000-1001** had knee surgery, **Subject 2760030-1002** did not reach 6 months, and **Subjects 8400184-1001 and 8400184-1002** did not reach 50 ED)
- One, **Subject 2760030-1005** completed the PK assessment but withdrew before treatment with AFSTYLA

¹ 1 Mixed Race, 2 Cape Coloured, and 1 White and Antillean MÃ©tis

In Trial CSLB627_3002:

- Two subjects were discontinued due to AEs that were not related to AFSTYLA
 - **Subject (b) (6)**, a 1 year old Caucasian male, was discontinued for a series of complex social circumstances, including Munchhausen syndrome by proxy.
 - **Subject (b) (6)**, a 10 year old Caucasian male, was discontinued for hip arthralgia.
- One subject was discontinued from the efficacy population due to a major protocol violation. **Subject (b) (6)**, a 10 year old Asian male, due to a laboratory screening error, had the screening sample reported as negative for pre-existing inhibitor when in fact it was positive. This subject was continued in the trial (prophylaxis arm) but was removed from the efficacy analysis.

Efficacy Analysis

On-demand treatment

In both trials, the primary outcome measure was response to treatment, assessed using the following scale:²

- **Excellent:** Definite pain relief and/or improvement in signs of bleeding within approximately 8 hours after 1 injection of AFSTYLA.
- **Good:** Definite pain relief and/or improvement in signs of bleeding at approximately 8 hours after the first AFSTYLA injection, but requires 2 injections for complete resolution.
- **Moderate:** Probable or slight beneficial effect within approximately 8 hours after the first AFSTYLA injection; requires more than 2 injections for complete resolution.
- **Poor/No response:** No improvement at all or condition worsens after the first AFSTYLA injection and additional hemostatic intervention is required with another FVIII product, cryoprecipitate, or plasma for complete resolution.

The primary endpoint for assessment of on-demand treatment of BE was the rate of treatment success, which was defined as a rating of ‘excellent’ or ‘good’ on the investigator’s overall clinical assessment of hemostatic efficacy 4-point scale.

On-demand treatment doses were based on the doses of FVIII products used prior to enrollment in the trials. The median dose per injection used to treat a BE was 31.7 (range: 6 to 84) IU/kg in Trial CSLB627_1001, and 27.3 (range: 16 to 76) IU/kg in Trial CSLB627_3002.

² This efficacy 4-point rating scale is a modification to a published scale (Tarantino MD, et al: Haemophilia, 2004;10:428-37) that has been used as a measurement of efficacy for multiple factor VIII products. The modification is a quantitative clarification of factor infusions to distinguish between “good” and “moderate” and was agreed upon by CSLB and FDA during the pre-IND period.

Overall in both trials, there were 1,261 BE and 1,195 of those required treatment (848 BE \geq 12 to 65 years and 347 BE <12 years). In subjects \geq 12 to 65 years, 94% of BE were controlled with 1 or 2 doses of AFSTYLA, and the overall rate of success (investigator's assessment of 'excellent' or 'good') was 92% (783/848 BE) with 95% CI (88.9%, 94.8%). In subjects <12 years, 96% of BE were controlled with 1 or 2 doses, and the overall rate of success was 96% (334/347 BE) with 95% CI (91.3, 98.4). The pre-specified success criterion for this endpoint (lower limit of the 95% CI > 70%) was met for both trials.

Routine prophylaxis

The primary outcome measure in Trial CSL627_1001 for routine prophylaxis was the annualized spontaneous bleeding rate (ASBR). The annualized bleeding rate (ABR) was a secondary outcome measure in Trial CSL627_3002.

In Trial CSL627_1001, dosing was individualized with a recommendation to start prophylaxis with a dose between 20 to 40 IU/kg every second day or 20 to 50 IU/kg 2 to 3 times/week; however, other schedules could be prescribed at the investigator's discretion taking into account the FVIII treatment regimen used prior to enrollment and the subject's bleeding phenotype. In Trial CSL627_3002, subjects could have received a dose of 15 to 50 IU/kg every second day, or 2 to 3 times per week, or at a dose and frequency determined by the investigator based on historical FVIII dosing and available PK data.

The median ASBR was 0 BE per year for all subjects in both trials. Forty-three percent of subjects in Trial CSL627_1001 and 26% of subjects in Trial CSL627_1001 receiving prophylaxis had no BE requiring treatment. In Trial CSL627_1001, when comparing between subjects on prophylaxis and subjects on on-demand treatment, routine prophylaxis decreased the ASBR³ by 92% (mean 1.6 vs. 19.5 respectively) and the ABR³ by 90% (mean 2.6 vs. 24.9 respectively). The ASBR in subjects in the prophylaxis group was significantly lower ($p < 0.0001$), than in the on-demand group.

In Trial CSL627_3002, a similar decrease in ABR³ (92%) – a secondary endpoint in this trial – with prophylactic treatment was reported. The mean ABR was 5.5 in the prophylaxis group and 71.5 in the 3 children in the on-demand treatment group. The median observed ABR by prophylaxis regimen was 2.3 with three times a week dosing and 4.4 with twice weekly dosing, and the percentage of subjects with no BE was higher in the three times a week group (37.5% versus 15%). The distribution of pediatric subjects in CSL627_3002 among the dosing regimens is shown in Table 7 below.

In Trial 627CSL_3002 the doses per injection were not different if subjects were treated twice or three times per week. Annual doses in these 2 groups were 4,117 versus 5,469 IU/kg/year, respectively. There were no age-related differences in AFSTYLA consumption. There was no reported shift to higher doses or more frequent dosing in Trial CSL627_3002, indicating that the doses recommended for routine prophylaxis in the label are appropriate.

³ Annualized number of BE, p-value and ratio are based on Poisson distribution.

Table 7: Dose Assignment, Efficacy Population, CSL627_3002

	On-demand (N = 3)	Every 2nd day (N = 3)	3 times/week (N = 24)	2 times/week (N = 43)	Other (N = 10)	Total (N=83)
Initial assigned dose	3	3	24	43	10	83
Final assigned dose	3	3	32	38	7	83
Mean final adjusted dose (range) IU/kg	25 (21 – 29)	44.3 (40 – 50)	34.9 (20 – 50)	36.2 (20 – 57)	43.1 (23 – 55)	36.2 (20 – 57)

Source: Modified from Recombinant coagulation Factor VIII, rFVIII, octagog alfa, Final Study Report Trial CSLB627_3002, 2016 February 4, Table 11-16, page 77 of 172

Surgical prophylaxis

Investigator’s (surgeon or anesthesiologist) assessment of expected blood loss compared to a hemostatically normal patient population using the following scale.⁴

- **Excellent:** Hemostasis clinically not significantly different from normal in the absence of other hemostatic intervention and estimated blood loss during surgery is not more than 20% higher than the predicted blood loss for the intended surgery.
- **Good:** Normal or mildly abnormal hemostasis in terms of quantity and/or quality, or estimated blood loss is >20% but ≤ 30% higher than the predicted blood loss for the intended surgery.
- **Moderate:** Moderately abnormal hemostasis in terms of quantity and/or quality with estimated blood loss greater than what is defined as ‘good’.
- **Poor/No response:** Severely abnormal hemostasis in terms of quantity and/or quality and/or additional hemostatic intervention required with another FVIII product, cryoprecipitate, or plasma for complete resolution.

Treatment success during the surgical sub-study was defined as an investigator rating of ‘excellent’ or ‘good’ on the 4-point efficacy evaluation of surgical treatment scale. The dose regimen for this part of the study was individualized based on the type of surgery and the clinical status of the subject. A major surgery was defined as a surgical procedure that involved anesthesia (general, spinal, epidural or regional block) or respiratory assistance (including but not limited to orthopedic and cardiac surgery).

The surgical sub-study was only a part of Trial CSLB627_1001. There were no surgical evaluations as a part of the design of Trial CSL627_3002. There were 16 reported surgeries in

⁴ This 4-point rating scale was agreed upon by CSLB and FDA during the pre-IND period and incorporates FDA’s suggestions.

13 subjects. All operations were major and non-emergency. The reported surgeries were oral surgery (n=1), abdominal surgery (n=2), orthopedic surgery (n=10) and circumcision (n=3). The investigators assessed hemostatic efficacy as “excellent” in 15 and “good” in 1, for an overall success rate of 100%; meeting the pre-specified success criterion rate of >70%.

Determination of FVIII Activity by the One-Stage Clotting and Chromogenic Substrate Assays

As discussed above in Section 3. Chemistry, Manufacturing and Controls (CMC) under a Product Quality/Potency, two assays are available to determine FVIII activity – the OS and ChS assays – and during the AFSTYLA clinical development program, reported FVIII activity levels determined by the OS assay were approximately 50% lower than those determined by the ChS assay. Although the magnitude of this discrepancy was consistent between subjects, these data were from CSLB’s central laboratory. Therefore, to investigate the potential variability in FVIII activity measurements in other laboratories outside of CSLB’s central laboratory, an international, multicenter, randomized and blinded field study was performed. The field study examined the relationship between the OS and ChS assays across the range of the assays.

The field study was performed by measuring FVIII activity in blinded samples of FVIII-depleted plasma, spiked with AFSTYLA, based on the chromogenic labeled potency, at (b) (4) [redacted]. These samples were distributed to participating laboratories to be assayed by the OS and ChS assays. (b) (4) [redacted] clinical laboratories – 13 from the United States – completed data entry. Laboratories performed FVIII activity assays on provided materials using standard reagents, and instrumentation that are employed routinely for FVIII activity measurement in their clinical hemostasis laboratories. All (b) (4) [redacted] laboratories evaluated the samples using an OS assay, and for 6 of the (b) (4) [redacted] the laboratories that routinely use it, the samples were also evaluated with the ChS assay.

(b) (4) [redacted]

[redacted]

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Based on these results a fixed conversion factor of 2 (i.e., the OS assay results multiplied by 2) can be used to align the OS and ChS assay results.

Bioresearch Monitoring

CBER conducted Bioresearch Monitoring (BIMO) inspections at three clinical trial sites that enrolled subjects for Trial CSLB627_1001 in support of this BLA. The three clinical sites selected for BIMO inspection represented approximately 19% of the subjects enrolled (33 out of 175). The BIMO inspections of three clinical investigators did not reveal substantive problems that impact the data submitted in the application.

b) Pediatric Study and PREA Requirements

AFSTYLA is a new active ingredient and triggers the Pediatric Research Equity Act (PREA), which requires AFSTYLA to be studied in children. Children ages 0 to <12 years of age were included in Trial CSLB627_3002. Adolescents ages 12 to <18 were included in Trial CSLB627_1001. The Pediatric Review Committee (PeRC) agreed with the Division's recommendation that the pediatric data submitted in this BLA are adequate for approval of AFSTYLA for a pediatric indication. Safe and effective perioperative use in children can be extrapolated from the adult data. No post-marketing pediatric studies are required for this product.

c) Other Special Populations

Only males were studied and no subjects were ≥ 65 years of age.

d) Overall Comparability Assessment

Not applicable.

7. Safety

Safety was assessed in both trials on the basis of the following variables:

- The nature and incidence of AEs
- The development of antibodies against AFSTYLA, antibodies to CHO proteins and inhibitors against FVIII
- Vital signs
- Laboratory safety parameters and the number and proportion of subjects with treatment-emergent abnormal laboratory values
- Clinically significant vital signs and changes from baseline in physical examination findings
- Clinical signs of thrombosis of any vessel as well as organ manifestations (e.g., myocardial infarction)
- Local tolerability

There were no deaths in either trial. One hundred and thirteen subjects in Trial CSLB627_1001 reported 292 treatment-emergent AE (TEAE), of which 13 subjects developed 19 TEAE considered related to AFSTYLA, and none resulted in withdrawal from the trial. In Trial CSLB627_3002, 50 subjects reported 113 TEAE, of which there were 11 serious TEAE (TESAE); all but 2 were mild or moderate in degree.

One TEAE – hypersensitivity reaction – in Trial CSLB627_1001 was considered related to AFSTYLA and one – positive inhibiting antibodies – in Trial CSLB627_3002 led to withdrawal from the trial. An additional TEAE – device occlusion – in Trial CSLB627_3002 was assessed by this reviewer to be related to AFSTYLA. These three TEAE are highlighted below.

- **Subject 6080002-1001** was a 17 year old Asian male. He had a history including hypersensitivity to cryoprecipitate, local hypersensitivity to a brand of adhesive dressing, and extrinsic asthma. This subject received 43 IU/kg of AFSTYLA as part of the prophylaxis treatment group. Approximately 2.5 hours after his last injection of AFSTYLA he developed severe pruritis, erythema of the hands and feet, chest pressure with dyspnea and severe headache. Upon admission to the emergency room there was no wheezing or stridor and he had good air entry. He was treated in the emergency room with steroids and antihistamines, and the episode resolved within approximately 17.5 hours after onset. He was discharged on the day of the event with a 5 day tapering course of steroids. He remained on AFSTYLA and tolerated it well thereafter without sequelae, and with no change in dose or treatment schedule. However, he did receive premedication prior to subsequent dosing. This hypersensitivity reaction was considered to be related to AFSTYLA.
- **Subject (b) (6)** : Development of inhibiting antibodies in a 10 year old Asian male in the prophylaxis arm was found to be due to a laboratory processing error. This subject's screening sample had been reported as negative when it was in fact positive for a preexisting inhibitor (3.46 BU/mL). By 4 months into treatment the inhibitor titer was negative (0.55 BU/mL) despite ongoing doses of AFSTYLA.
- **Subject (b) (6)** : A device occlusion in a 6 year old Caucasian male in the prophylaxis arm was assessed as unrelated to AFSTYLA by CSLB but was assessed as possibly related by the clinical reviewer. A Port-a-Cath® indwelling central venous access catheter developed an intramural thrombus 5 weeks into treatment. This device had been in situ for 3 years.

Severe TEAE – both unrelated to AFSTYLA – are noted below.

- **Subject (b) (6)** : 4 year old Asian in the prophylaxis arm developed systemic inflammatory response syndrome after parents flushed study medication through a catheter with unsterile saline.
- **Subject (b) (6)** : 7 year old Caucasian male in the prophylaxis arm developed a traumatic splenic rupture.

Immunogenicity

Neutralizing antibodies:

- Inhibitor formation was not detected in any subject in either trial including those subjects with ≥ 50 ED.

Binding antibodies:

- Of eight subjects in trial CSLB627_1001 who had non-inhibitory anti-drug antibodies present at baseline, seven remained positive until the end of the trial. Four subjects developed non-inhibitory IgG and/or IgM antibodies during the trial. Two of these became negative by the end of the trial. No subject in this trial had or developed anti-CHO antibodies. In Trial CSLB627_3002, 10 subjects had non-inhibitory antibody on enrollment and 10 subjects developed non-inhibitor antibodies during the trial. Of these 10, three had negative results at the end-of-study and seven had a positive result. None developed any related symptoms. Actual titers and IgG subclasses were not reported for non-inhibitory anti-drug antibodies, and PK studies were not repeated to exclude an effect of non-inhibitory antibodies on clearance of AFSTYLA.
- No subject in either trial had or developed antibodies to CHO host cell proteins.

Clinical Laboratory Evaluations

There were no clinically relevant changes in laboratory values considered to be related to AFSTYLA by either the applicant or the clinical reviewer.

Physical Assessments and Vital Signs

The clinical reviewer found no concerns regarding trends in vital signs observed after infusions of AFSTYLA.

AEs of special interest

- Thromboembolic events (TEE)

There was no report of TEE in trial CSLB627_1001. In trial CSLB627_3002 CSLB reported no TEE. However the clinical reviewer assessed one event as possibly related. **Subject (b) (6)**, a 6 year old Caucasian, developed an intramural thrombus of a Port-a-Cath® indwelling venous access catheter five weeks into treatment.

- Hypersensitivity reactions

There were 14 reports of possible hypersensitivity reactions (1.1%) in trial CSLB627_1001. Upon review, 11 were excluded for mild and nonspecific signs, leaving four considered by the clinical reviewer as hypersensitivity reactions:

- **Subject 6080001-1002**, a 32 year old Asian male with 2 related non-serious TEAEs of hypersensitivity. The dose of study drug was unchanged for both events. After the second event the subject was advised to remain in the clinic for observation for 1 hour after AFSTYLA injection. The subject recovered from both events.
- **Subject 6080001-1003**, a 23 year old Asian male with one related non-serious TEAE of erythema. The dose of AFSTYLA was unchanged, no other treatment was given. The subject recovered.
- **Subject 8400154-1004**, a 21 year old Caucasian male with one related non-serious TEAE of rash. AFSTYLA infusion was interrupted due to this event, but no other action was taken, and the subject was recovering at the time of

reporting. It is possible that this bilateral hand rash was related to a new job that required the use of latex gloves.

In Trial CSLB627_3002 13 subjects developed 19 events suggestive of a hypersensitivity reaction. All but one was considered unrelated to the study drug.

- **Subject (b) (6)**, a 9 year old Asian experienced a single event of hypersensitivity considered mild and the dose of AFSTYLA was not altered.

Symptoms for all hypersensitivity reactions were mild and treatment was continued without ill effect.

Conclusions

AFSTYLA demonstrated adequate efficacy with an acceptable safety profile in adult and pediatric patients with hemophilia A. The clinical reviewer recommends approval for the indications of on-demand treatment and control of BE, routine prophylaxis to reduce the frequency of BE, and perioperative management of bleeding, in children and adults with hemophilia A (congenital Factor VIII deficiency).

8. Advisory Committee Meeting

The Division of Hematology Research and Review and the Division of Hematology Clinical Review in the Office of Blood Research and Review reviewed the information in this application and determined that referral to the Blood Products Advisory Committee prior to product approval was not needed for the following reasons (FDAAA [HR 3580-138 SEC. 918: REFERRAL TO ADVISORY COMMITTEE]):

- The new molecular entity (NME) provision does not apply to AFSTYLA because it does not represent a novel product class. Recombinant FVIII products have been licensed in the United States since 1992 and have been used to control and prevent bleeding in patients with hemophilia A. The first product in this class, RECOMBINATE, was approved by the FDA in 1992, and currently there are several full-length and B-domain-deleted FVIII products licensed in the United States.
- The mechanism of action of FVIII and its function in blood coagulation are well studied and understood. Upon activation of FVIII by thrombin, FVIIIa acts as a cofactor for activated Factor IX triggering a chain of biochemical reactions – activation of Factor X, which converts prothrombin into thrombin, and subsequent interaction of thrombin with fibrinogen results in the formation of a fibrin clot that stops bleeding. When administered to a patient with hemophilia A, FVIII products temporarily replace the missing or defective endogenous FVIII.
- The amino acid sequence of AFSTYLA is based on that of a B-domain-deleted human FVIII, with only minor modifications, which are related to the removal of a processing site in the acidic region of the a3 domain. The resulting single-chain molecule

possesses the same functional characteristics as other FVIII molecules. As noted above, after activation by thrombin and removal of the (b) (4) B- and a3-domain, the activated rFVIII (rFVIIIa) molecule formed has an amino acid sequence identical to FVIIIa formed from endogenous, full-length FVIII. Consequently, the hemostatic activity of AFSTYLA is consistent with that of other licensed FVIII products and enables the formation of a fibrin clot via the intrinsic coagulation pathway.

- The manufacturing process for AFSTYLA includes two viral clearance steps – (b) (4) [REDACTED] for virus removal – that meet the current requirements for assuring product safety with regard to adventitious viruses.
- The proposed indications for AFSTYLA are similar to those of other U.S. licensed FVIII products.
- The design of the pivotal clinical study to evaluate the safety and efficacy of AFSTYLA was adequate, and the results of the studies did not raise any concerns.
- Review of information submitted in the BLA for AFSTYLA did not raise any controversial issues or pose unanswered scientific questions which would have benefited from Advisory Committee discussion and recommendations. The sole significant issue was related to the use of a chromogenic substrate assay for AFSTYLA potency assignment, and was resolved at the Office level.

9. Other Relevant Regulatory Issues

The following regulatory actions were considered to minimize the risk for unnecessary additional dosing, higher chronic dosing, or investigations for an inhibitor due to incorrect interpretation of the Factor VIII activity levels obtained by the OS assay:

- A Postmarketing Requirement (PMR) or Risk Evaluation and Mitigation Strategy (REMS) with or without Elements to Assure Safe Use to ensure that the benefits of AFSTYLA outweigh the risks of misinterpretation of OS assay results
- Request CSLB to devise and implement a communication plan to educate, inform, and raise awareness of risk (e.g., Dear Healthcare Provider Letter [DHCP] and disseminating information through professional societies)
- Describe the issue in the AFSTYLA labeling (PI), and use a boxed warning to have the message of the risk made ubiquitous

The review team determined that a PMR or REMS was not warranted because the most likely clinically relevant risk to patients is overdosing with AFSTYLA, which is unlikely to cause harm as doses in excess of 100 IU/kg (twice the highest proposed labeled dose) were administered in Trial CSL627_1001 without report of thrombotic events. Further, the results of the field study and the PK data generated from the product development program indicate that the relationship between the two assay methods offers the ability to multiply a OS assay result by a conversion factor of 2 to determine the patient's Factor VIII activity level. Additionally,

the review team determined that issue did not rise to the level to meet the regulatory requirements of a boxed warning.

Therefore, the following communication strategies will be put in place to warn treating physicians of the issue:

1. The issue was described in the PI. The following language was included in WARNINGS AND PRECAUTIONS subsection 5.3 Monitoring Laboratory Tests:

Monitor plasma Factor VIII activity in patients receiving AFSTYLA using either the chromogenic assay or the one stage clotting assay, which is routinely used in US clinical laboratories. The chromogenic assay result most accurately reflects the clinical hemostatic potential of AFSTYLA and is preferred. The one-stage clotting assay result underestimates the Factor VIII activity level compared to the chromogenic assay result by approximately one-half. If the one stage clotting assay is used, multiply the result by a conversion factor of 2 to determine the patient's Factor VIII activity level. Incorrect interpretation of the Factor VIII activity obtained by the one stage clotting assay could lead to unnecessary additional dosing, higher chronic dosing, or investigations for an inhibitor.

2. CSLB will implement a multifaceted communication plan to include:
 - a. An Important Prescribing Information DHCP letter related to laboratory monitoring tests, in accordance with the FDA guidance, to be distributed to hematologists within 60 days of the BLA approval. The DHCP letter includes a contact number for CSL Behring Medical Information phone-line if additional guidance is needed regarding interpretation of FVIII activity measurements obtained using the OS assay in patients receiving AFSTYLA.
 - b. Outreach targeted at hematologists and pathologists to include:
 - i. Scientific communications in journals and at professional society meetings, peer-to-peer education speaker programs, and a webinar with case studies
 - ii. Sales force communications with handouts/communications that will include hospitals' and hematology treatment centers' formulary committee members
 - iii. Dedicated section of the product's website to address the issue
 - iv. Key word optimization to allow for search engines to find assay information
 - v. Use of focus groups to inform the development of educational materials
 - vi. Include information related to the assay conversion factor in fair balance statements

The review team, in consultation with a special government employee expert hematologist, assessed the above communication strategies, agreed upon by CSLB, and concluded that they are adequate to ensure that AFSTYLA's benefits outweigh its risks.

10. Labeling

The proposed proprietary name for the product, AFSTYLA, was reviewed by the Advertising and Promotional Labeling Branch (APLB) and recommended to be acceptable on July 20, 2015. The product labeling (i.e., prescribing information, patient package insert, and instructions for use) and the product package and container labels were reviewed, commented, and/or revised by the appropriate discipline reviewers before APLB conducted its review from a promotional and comprehension perspective.

FDA comments and recommendations regarding the product labeling and labels were initially conveyed to CSLB on April 26, 2015 and negotiated throughout the month of May, 2016.

FDA had multiple interactions with the applicant regarding the recommendation that a conversion factor of (b) (4) 2 be used to determine FVIII activity when the OS assay is used. FDA deemed that either was adequate and appropriate. Following these interactions, CSLB selected a conversion factor of 2 due to relative ease of use compared to (b) (4)

11. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The CBER review committee recommends approval of this BLA. The manufacturing process for AFSTYLA was found validated and adequately controlled. Clinical efficacy and safety data for AFSTYLA support a favorable benefit/risk determination for the proposed indications in children and adults with hemophilia A (congenital Factor VIII deficiency) for:

- On demand treatment and control of bleeding episodes
- Routine prophylaxis to reduce the frequency of bleeding episodes
- Perioperative management of bleeding

b) Risk/Benefit Assessment

Hemophilia A patients are at risk of acute bleeding episodes predominantly into joints, muscles, mucosa, and body cavities. Repeated bleeding into a joint can lead to disabling joint disease. AFSTYLA replaces the missing FVIII that is needed to achieve hemostasis in patients with hemophilia A. In recent years, treatment regimens have shifted from on-demand treatment of BE to routine prophylaxis due to observed improvement in long-term clinical outcomes such as joint damage. AFSTYLA is a rFVIII produced in a CHO cell line. The cell line utilizes (b) (4) media entirely devoid of animal- or human-derived materials. Truncation and partial deletion of the molecule allow its expression as a covalently bonded single chain.

Benefits

The efficacy of AFSTYLA has been established for on-demand treatment and control of bleeding episodes, perioperative management of bleeding, and routine prophylaxis to reduce

the frequency of bleeding episodes, using data from two “pivotal” trials. Two hundred and fifty-eight adult and pediatric subjects complied with the trial protocols. They experienced 963 BE requiring treatment with AFSTYLA, and subjects underwent 16 surgical procedures.

Risks

Potential safety concerns for AFSTYLA include hypersensitivity reactions, the development of neutralizing antibodies to FVIII, and thromboembolic events. Hypersensitivity reactions have been reported with both plasma-derived and recombinant FVIII products and can manifest as anaphylactic or allergic reactions.

There was a single serious hypersensitivity reaction in an adult subject and two mild reactions in an adult and two children that did not persist with continued treatment with AFSTYLA. Inhibitor formation was not detected in any subject in either trial. Even though no subjects developed neutralizing antibodies to FVIII, the potential for developing inhibitors is included in the Warnings and Precautions section of the Package Insert. No TEE were reported in trial CSLB627_1001. There was a single event of intraluminal thrombosis of a long term indwelling venous catheter in trial CSLB627-3002 that this reviewer assessed as possibly related.

There is potential for misinterpretation of reported FVIII activity levels during monitoring of patients receiving AFSTYLA because the two available clinical assays report disparate results. The results determined by the OS assay, routinely used in US clinical laboratories, are approximately 50% lower than those determined by the ChS assay. Lack of correction with a conversion factor to bring the results into alignment could lead to unnecessary additional doses, to higher chronic dosing (as in prophylactic therapy), or to unnecessary investigations for an inhibitor. An element of the risk for patients whose healthcare providers rely on the OS assay for FVIII activity levels will depend on the individual healthcare provider’s understanding of how to interpret the OS assay results.

CSLB recognizes the importance of ensuring that healthcare providers are well informed about dosing and monitoring of FVIII activity levels of patients receiving AFSTYLA to ensure positive clinical outcomes. Therefore, direction on the interpretation of OS and ChS assay results for patients treated with AFSTYLA is provided in WARNINGS AND PRECAUTIONS Section 5.3 (Monitoring Laboratory Tests) of the label. Additionally, CSLB has committed to carry out a multi-faceted communication plan (see Section 9. Other Relevant Regulatory Issues), designed to educate healthcare providers about the use and interpretation of laboratory assays to monitor patients treated with AFSTYLA. The communication strategies that comprise this communication plan have been added to the AFSTYLA pharmacovigilance plan.

Overall Benefit/Risk Profile

The overall benefit/risk profile of AFSTYLA is favorable. Clinical studies demonstrated efficacy of AFSTYLA for its labeled indications.

c) Recommendation for Postmarketing Risk Management Activities

See Section 9. Other Relevant Regulatory Issues for details of the agreed upon communication strategies to educate, inform, and raise awareness of the risk of misinterpretation of FVIII activity levels in patients treated with AFSTYLA and whose FVIII activity levels are monitored using the OS assay.

d) Recommendation for Postmarketing Activities

CSLB made the following post-marketing commitments:

- i) CSLB commits to assess data after producing ^{(b) (4)} commercial scale GMP batches to revise the acceptance criteria of the (b) (4) CSLB commits to perform an interim statistical re-assessment of the alert limits after evaluating commercial scale GMP batches manufactured by May 31, 2017 and submit the interim report as a Changes Being Effected **Supplement contains PMR/PMC Submission – Final Study Report** by July 31, 2017 and submit the final acceptance criteria as a Prior Approval **Supplement contains PMR/PMC Submission – Final Study Report** by September 30, 2018.
- ii) CSLB commits to investigate the (b) (4) and agrees to submit a Supplement containing the Postmarketing Commitment –Final Study Report by May 31, 2017.
- iii) CSLB commits to develop and validate an (b) (4) method in which the (b) (4), and agrees to submit a Supplement containing the Postmarketing Commitment - Final Study Report by May 31, 2017.