CLINICAL STUDY PROTOCOL

A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A

Study Number:	CSL627_3001
Study Product:	rVIII-SingleChain, CSL627
Development Phase:	Phase 3
Sponsor:	CSL Behring GmbH
Protocol Version:	Amendment 5, FINAL
EudraCT Number:	2013-003262-13
IND Number:	CCI [redacted]
Protocol Date:	26 May 2020
Compliance:	This study will be conducted in accordance with standards of Good Clinical Practice (as defined by the International Conference on Harmonisation), ethical principles that have their origin in the Declaration of Helsinki and all applicable national and local regulations.

List of Personnel and Organizations Responsible for Conduct of the Study

A list of personnel and organizations responsible for the conduct of the study will be supplied to study sites as part of the Investigator's Study File. This list will be updated by CSL (or delegate) and provided to the study sites as needed.

Protocol Synopsis

Title	A Phase 3 Open Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A
Study Number	CSL627_3001
Sponsor	CSL Behring GmbH
Development Phase	Phase 3
Study Product	rVIII-SingleChain, CSL627
Indication	Hemophilia A
Study Summary	This multicenter, open-label, phase 3 extension study will investigate the safety and efficacy of rVIII-SingleChain for prophylaxis and on-demand treatment of bleeding episodes in a total of at least 224 subjects with severe congenital hemophilia A. The study enrolls both subjects with and without previous exposure to factor VIII (FVIII) replacement therapy in 1 of the following 3 arms: 1. **Arm 1** (previously treated patients [PTPs]): subjects of all ages who have participated in a previous CSL Behring (CSL)-sponsored study with rVIII-SingleChain. 2. **Arm 2** (previously untreated patients [PUPs]): subjects 0 to < 18 years of age who have not been exposed previously to any FVIII product. 3. **Arm 3:** PTPs < 65 years of age with at least 50 exposure days (EDs) to any FVIII product and not currently enrolled in a rVIII-SingleChain study. A surgery substudy (open to all study arms) will investigate the use of rVIII-SingleChain in surgery. CCI [redacted]

Primary Objectives	• **Arm 1 and Arm 3 (PTPs)**: to characterize the long-term safety profile of rVIII-SingleChain with respect to inhibitor development in PTPs.
	• **Arm 2 (PUPs)**:
	  • To characterize the safety with respect to inhibitor development in PUPs.
	  • To evaluate the efficacy of on-demand and prophylaxis treatment of rVIII-SingleChain in PUPs.

Primary Endpoints	• **Arm 1 and Arm 3 (PTPs)**: incidence of inhibitor formation to FVIII in at least 200 PTPs with at least 100 EDs of rVIII-SingleChain
	• **Arm 2 (PUPs):**
	  • Incidence of high-titer inhibitor formation to FVIII (ie, inhibitor titer of ≥ 5 Bethesda units [BU]/mL) in PUPs with at least 50 EDs of rVIII-SingleChain.
	  • Treatment success for **major** bleeding episodes, defined as a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale.
	  • Annualized spontaneous bleeding rate (AsBR) during prophylaxis and on-demand treatment.

Secondary Objectives	• **Arm 1 and Arm 3 (PTPs)**:
	  • To characterize the safety profile of rVIII-SingleChain with respect to inhibitor development after 10 EDs and after 50 EDs.
	  • To characterize the safety profile of rVIII-SingleChain with respect to antibodies against rVIII-SingleChain and antibodies to Chinese hamster ovary (CHO) proteins.
	  • To collect and evaluate efficacy information on the prophylaxis and treatment of bleeding episodes.
	  • To assess the hemostatic efficacy of rVIII-SingleChain for PTPs who undergo surgery, using the 4-point efficacy evaluation of surgical treatment scale.
	• **Arm 2 (PUPs)**:
	  • To further characterize the safety profile of rVIII-SingleChain with respect to inhibitor development.
	  • To characterize the safety profile of rVIII-SingleChain with respect to antibodies against rVIII-SingleChain and antibodies to CHO proteins.
	  • To collect and evaluate the number of rVIII-SingleChain injections required for the treatment of bleeding episodes.
	  • To characterize consumption of rVIII-SingleChain in prophylaxis, on-demand treatment, and surgery.
	  • To assess the hemostatic efficacy of rVIII-SingleChain for PUPs who undergo surgery, using the 4-point efficacy evaluation of surgical treatment scale.
	  • To assess the occurrence of clinically significant abnormalities in vital signs after rVIII-SingleChain administration.

Secondary Endpoints	• **Arm 1 and Arm 3 (PTPs)**:
	  • Incidence of inhibitor formation to FVIII in at least 200 PTPs after 10 EDs and after 50 EDs with rVIII-SingleChain
	  • Percentage of PTPs who develop antibodies against rVIII-SingleChain.
	  • Percentage of PTPs who develop antibodies to CHO proteins.

- Annualized bleeding rate (ABR) (traumatic and non-traumatic) during prophylaxis and on-demand treatment.
- Treatment success for bleeding episodes defined as a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale.
- Percentage of bleeding episodes requiring 1, 2, 3, or > 3 injections of rVIII-SingleChain to achieve hemostasis.
- Mean actual dose per kg per subject per year; consumption of rVIII-SingleChain, expressed as number of injections and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).
- Treatment success for surgery, using the 4-point efficacy evaluation of surgical treatment scale.

- **Arm 2 (PUPs)**:
  - Incidence of high-titer inhibitor formation to FVIII (ie, inhibitor titer of ≥ 5 BU/mL) after 10 EDs with rVIII-SingleChain in PUPs.
  - Incidence of low-titer inhibitor formation (ie, inhibitor titer of < 5 BU/mL) to FVIII after 10 EDs and after 50 EDs with rVIII-SingleChain in PUPs.
  - Incidence of total (low- and high-titer) inhibitor formation to FVIII after 10 EDs and after 50 EDs with rVIII-SingleChain in PUPs.
  - Percentage of PUPs who develop antibodies against rVIII-SingleChain.
  - Percentage of PUPs who develop antibodies to CHO proteins.
  - Treatment success for **non-major** bleeding episodes, defined as a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale.
  - Percentage of bleeding episodes requiring 1, 2, 3 or > 3 injections of rVIII-SingleChain to achieve hemostasis.
  - ABR during prophylaxis and on-demand treatment.
  - Mean actual dose per kg per subject per year; consumption of rVIII-SingleChain, expressed as number of injections and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).
  - Treatment success for surgery, using the 4-point efficacy evaluation of surgical treatment scale.
  - Percentage of PUPs with clinically significant abnormal values for blood pressure, or heart rate, or body temperature at 1, 2, 3, or 6 hours after the first rVIII-SingleChain injection.
  - Percentage of PUPs with treatment-emergent clinically significant abnormal values for blood pressure, heart rate or body temperature during the course of the study.

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Safety Variables	• **All study arms**:
	  • Frequency, severity and seriousness of adverse events (AEs).
	  • Laboratory values over time, individual subject changes, and clinically significant abnormal values.
	  • Vital signs values over time, individual subject changes, and clinically significant abnormal values.
	  • Physical examination.
	  • Subject / caregiver assessment of local tolerability.
	• **Arms 2 and 3 only**: investigator assessment of local tolerability (for rVIII-SingleChain injections administered at the clinic).

Study Design	Multicenter, non-randomized, open-label, multiple-arm, phase 3 study.
Number of Subjects	A total of at least 224 subjects will be enrolled in this study. Of these, at least 200 PTPs will be in Arms 1 and 3 combined, and at least 24 PUPs will be in Arm 2.

Study Duration	**Arm 1 and Arm 3 (PTPs)**: Each PTP will stay in this study until they have achieved at least 100 EDs, which is expected to take up to 5 years. **Arm 2 (PUPs)**: Inhibitor-negative PUPs will stay in this study until they have achieved 75 EDs. Additional time in the study may occur for PUPs that develop an inhibitor: • CCI [redacted] • For inhibitor-positive PUPs who remain in the **main study** who have achieved eradication, the End of Study Visit will occur when they complete the 12-month post eradication follow-up period in the main study. The maximum duration of the inhibitor treatment period is 24 months starting when the dose is initially adjusted to treat the inhibitor. If no dose adjustment is performed to treat the inhibitor then the duration of inhibitor treatment will be from the date of inhibitor diagnosis.
Study Population and Main Criteria for Eligibility	All eligible subjects will be males who have been diagnosed with severe congenital hemophilia A (FVIII activity levels < 1%, determined in local laboratory before enrollment or documented in subject's medical record). **Main inclusion criteria:** • **Arm 1 (PTPs)**: subjects of any age who have participated in a previous CSL-sponsored clinical study with rVIII-SingleChain. • **Arm 2 (PUPs):** subjects 0 to < 18 years of age who have not been exposed to any prior FVIII product (except for short-term use of blood products). • **Arm 3 (PTPs):** subjects 0 to < 65 years of age who have had at least 50 EDs to a prior FVIII product and who are not currently enrolled in a rVIII-SingleChain study. **Main exclusion criteria:** • Known or suspected hypersensitivity to rVIII-SingleChain, to any excipients of rVIII-SingleChain, or to CHO proteins. • Currently receiving a therapy not permitted during the study. • Serum creatinine > 2 times the upper limit of normal (× ULN), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5 × ULN at Screening (if specified for a particular arm). • Any first-order family (eg, siblings) history of FVIII inhibitors. • **Arm 3**: Any history of or current FVIII inhibitors.

	CCI [redacted]
Study Product, Dose, Dosing Regimen and Administration	In all study arms, the investigator will assign subjects to either prophylaxis or on-demand treatment regimens with rVIII-SingleChain. All subjects will be dosed according to the actual potency printed on the vial label and will be treated by IV injection. On-demand and prophylaxis doses can be adjusted as necessary at the discretion of the investigator. **Prophylaxis treatment regimen:** In previous studies, most subjects received prophylaxis treatment with an initial range of 15 to 50 IU/kg rVIII-SingleChain every second day or 2 to 3 times per week. In all study arms, the dose prescribed will be based on the subject's weight at the most recent visit. • In **Arm 1 and Arm 3 (PTPs)**, the investigator will determine the rVIII-SingleChain prophylaxis dose and dosing schedule for the subject based upon the subject's pharmacokinetic (PK) profile, rVIII-SingleChain PK data, previous FVIII treatment regimen, bleeding phenotype (if available), and taking into consideration the World Federation of Hemophilia (WFH) guidelines [WFH, 2012]. • In **Arm 2 (PUPs),** the investigator will determine the rVIII-SingleChain prophylaxis dose and dosing schedule at their discretion, taking into consideration the WFH guidelines [WFH, 2012], subject's age and other disease characteristics (eg, bleeding phenotype). **Treatment of bleeding episodes:** In the event of a bleeding episode, subjects will be treated at a dose pre-determined by the investigator based on the type and severity of the bleeding episode. All subjects should treat bleeding episodes with rVIII-SingleChain when they occur, regardless of the assigned treatment regimen. The desired FVIII level for the treatment of a bleeding episode (on-demand treatment) is based on the recommendations of the WFH [WFH, 2012]. CCI [redacted]
Comparator Product, Dose, Dosing Regimen and Administration	Not applicable.
Efficacy Assessments	In all study arms, efficacy assessment will be based on total consumption of rVIII-SingleChain, number of bleeding episodes, investigator's clinical assessment for hemostatic efficacy (4-point scale) for treatment of bleeding episodes and for surgery, annualized bleeding rate, and number of rVIII-SingleChain injections required to achieve hemostasis.

Safety Assessments	In **Arm 1 and 3 (PTPs),** inhibitor development will be assessed at the closest visits after 10, 50, and 100 EDs. In **Arm 2 (PUPs)**, inhibitor development will be assessed regularly based on treatment regimen assigned as detailed in the Schedules of Assessments below. An inhibitor test prior to surgery is recommended for PUPs undergoing surgery. CCI [redacted] Additional safety assessments include AEs, serious AEs (SAEs), laboratory values, physical examinations, vital signs, antibodies against rVIII-SingleChain and CHO proteins, and local tolerability (subject / caregiver assessment in all study arms, investigator assessment for site injections only in Arms 2 and 3). As part of the safety assessment, incremental recovery (IR) will be monitored. CCI [redacted]
Pharmacokinetics	In **Arms 1 and 3 (PTPs)**, IR will be assessed at Day 1 and at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs (Arm 1: cumulative EDs from first dose of rVIII-SingleChain in the parent study; Arm 3: EDs from first dose of rVIII-SingleChain in the present study). CCI [redacted]
Pharmacodynamics	Not applicable.
Other Assessments	Not applicable.
Statistical Analyses	The incidence of inhibitor formation to FVIII over 100 EDs of rVIII-SingleChain in PTPs (Arm 1 and Arm 3) and the incidence of high-titer inhibitor formation over 50 EDs of rVIII-SingleChain in PUPs (Arm 2) will be reported together with two-sided 95% confidence intervals (CIs). In Arm 2 (PUPs), treatment success of **major** bleeding episodes (defined as the percentage of bleeding episodes with a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale) will be reported together with a two-sided 95% CI. A generalized linear model will be used to account for within-subject correlation. AsBR during prophylaxis and on-demand will be summarized using descriptive statistics. In addition, the number of spontaneous bleeding

	episodes per year and associated 95% CI will be estimated based on a Poisson model. Secondary and CCI endpoints will be summarized using descriptive statistics, or frequency counts and percentages. Descriptive statistics will include number of observations, mean, standard deviation, median, minimum and maximum; and additional descriptive statistics (eg, quartiles, coefficient of variation) may be reported when appropriate. All data will be analyzed and presented separately for PTPs and PUPs as well as for the surgery and CCI substudies.
Interim Analyses	No formal interim analyses are planned. The final analysis of PTPs will be conducted when all PTPs have completed the study to support the production of a study report on PTPs before the reporting for PUPs. In addition, safety, efficacy and / or PK data may be reported to regulatory authorities while the study is ongoing to supplement information from completed lead-in studies or the ongoing study. Those analyses do not have an impact on the overall study design or further study conduct, and therefore are not considered to be interim analysis in the sense of International Conference on Harmonisation (ICH) E9.

Schedule of Assessments:

Schedule of Assessments for Arm 1 (PTPs from rVIII-SingleChain Study)

Assessments / **Visits**	**Day 1**	**Every 3 months[A] (± 7 days)**	**EOS[B]**
Informed consent	X		
Demographic information	X[C]		
Medical and surgical history	X[C]		
Review of inclusion / exclusion criteria; subject eligibility	X		
Hemophilia A history (previous FVIII treatment, blood group, and gene defect)	X[C]		
Hemophilia A social history and activity level	X[C]	X	X
Blood sample for gene defect, if no medical record	← *At any time during the study*[D] →		
Serum chemistry and hematology[E,F]	X[C]	X	
CD4 lymphocyte count (for HIV+ subjects)	X		
Vital signs[F]	X[C]	X	
Physical examination[F]	X[C]	X	
Body weight and height[G]	X	X	
Incremental recovery[H]	X	←------*At specified timepoints*---→	
Inhibitor to FVIII[I,J]	X[C]	←-----*At specified timepoints*----→	X
Antibodies against rVIII-SingleChain and CHO proteins[I,J]	X[C]	←-----*At specified timepoints*----→	
Virology retention sample[K]	X[C]		X
Distribute and train subject on eDiary	X		
Review subject eDiary		X	X
Collect subject eDiary			X
Assign treatment regimen	X		
Distribute rVIII-SingleChain	X	X[L]	
Record investigator assessment for treatment efficacy		X	X
Collect used and partially used vials of rVIII-SingleChain[M]		X	X
Drug accountability		X	X
Adverse events[N]	←----------*On an ongoing basis*----------→		
Concomitant medications	←----------*On an ongoing basis*----------→		

Abbreviations: CD4 = cluster of differentiation 4; CHO = Chinese hamster ovary; eCRF = electronic case report form; ED = exposure day; eDiary = electronic diary; EOS = End of Study; FVIII = coagulation factor VIII; HIV = human immunodeficiency virus; IR = incremental recovery; PTP = previously treated patient; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments:

A: One month is 28 days.

B: The EOS visit should take place at the end of the planned treatment period, if the subject discontinues prematurely, or if the sponsor terminates the study.

C: Data will be acquired from the EOS visit from previous CSL-sponsored rVIII-SingleChain studies for Day 1 assessments and will include demographic information, medical and surgical history, hemophilia A history, hemophilia A social history and activity level, serum chemistry, hematology, vital signs, physical examination, blood sample for inhibitors and antibodies, virology retention sample, adverse events, and concomitant therapy, if available. If not available, these assessments will be performed at the Day 1 visit.

D: If no medical record. Blood sample for gene defect should preferably be taken at Day 1. If this is not possible, sample can be taken at any time during study.

E: Hematology: hemoglobin, hematocrit, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and platelet count.
Serum chemistry: creatinine, alanine aminotransferase, aspartate aminotransferase, and total bilirubin.

F: Hematology, serum chemistry, physical examinations, and vital signs will be performed during every site visit until the subject reaches 50 EDs, then yearly until EOS.

G: Body weight will be measured for all subjects during each site visit. Height will be measured at each site visit only for subjects < 18 years old.

H: Blood samples will be collected to assess IR and be analyzed in the central laboratory. If possible, subjects should have a 4-day washout after last FVIII injection before blood draws. A pre-dose blood sample will be drawn. Subjects will then receive their prophylaxis dose of rVIII-SingleChain injection in the clinic. For on-demand subjects, the usual dose used to treat a bleeding episode or any other dose determined by the investigator will be administered in the clinic. A post-dose blood sample will be drawn 30 to 60 minutes (± 5 minutes) after the injection. Incremental recovery will be collected at Day 1 and at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs (see Section 8.6.2). Exposure days are cumulative from previous lead-in studies (calculated from the first dose of rVIII-SingleChain in any study.

I: Data will be acquired from the EOS visit from previous CSL-sponsored rVIII-SingleChain studies for inhibitors to FVIII, antibodies against rVIII-SingleChain and antibodies against CHO proteins for Day 1. Additional blood samples for inhibitors to FVIII and antibodies to rVIII-SingleChain and antibodies to CHO proteins will be collected at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs. For the inhibitor blood samples, subjects should have a 4-day washout from last FVIII injection before blood draw if possible.

J: If the investigator collects blood samples for FVIII levels or inhibitor assessment at any other time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory (see Section 8.6).

K: Virology retention sample: CSL will retain a sample for 5 years from EOS.

L: rVIII-SingleChain may be dispensed at more frequent schedules if need is determined by the investigator.

M: Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at the next scheduled visit. All unused vials will be returned at the EOS visit.

N: The observation period for adverse event (and serious adverse event) reporting in an individual subject will start at the time of giving written informed consent for participation in the current study and finish with the EOS visit. However, adverse events ongoing at the EOS visit will be followed until resolution or 28 days after the final administration of rVIII-SingleChain during this study, whichever is sooner.

Schedule of Assessments for Arm 2 PUPs With or Without Bleeding Episode at Screening and Assigned to PROPHYLAXIS

Assessments / Visits	Screening (Up to 28 days before Visit 1)[A]	Visit 1[A]	Every month (± 7 days): Until 25 EDs achieved[B,C,D,E]	Every 3 months (± 7 days): From 25 EDs until EOS[B,C,D,E]	EOS[E]
Informed consent / assent	X				
Demographic information	X				
Medical and surgical history	X				
Review of inclusion / exclusion criteria; subject eligibility	X	X[F]			
Hemophilia A history (blood group, and gene defect)	X				
Hemophilia A social history and activity level	X	X	X	X	X
Review of prior medications (previous 28 days)	X				
Blood sample for gene defect, if no medical record[G]		← *At any time during study*[G] →			
Serum chemistry and hematology[H]	X		X	X	X
CD4 lymphocyte count (for HIV+ subjects)[I]	X				
Vital signs	X	X[J]	X	X	X
Physical examination	X		X	X	X
Body weight and height	X	X	X	X	X
Inhibitor to FVIII[K,L]	X		X[S]	X[S]	X
Antibodies against rVIII-SingleChain and CHO proteins [K,L]	X		X	X	X
Plasma FVIII activity	X[M]				
Virology retention sample		X[N]			X
Distribute and train subject on eDiary		X			
Review subject eDiary and collect at EOS visit			X	X	X
Administer first rVIII-SingleChain dose at clinic[O]		**X[O]**			
Local tolerability (if rVIII-SingleChain administered at clinic)		X	X	X	
Record investigator assessment for treatment efficacy		X	X	X	X
Assign treatment regimen		X	X[P]	X[P]	
Distribute rVIII-SingleChain		X	X	X	
Collect used and partially used vials of rVIII-SingleChain[Q]			X	X	X
Perform drug accountability			X	X	X

Assessments / **Visits**	**Screening (Up to 28 days before Visit 1)A**
Adverse events[R]	←----------*On an ongoing basis*----------→
Concomitant medications	←----------*On an ongoing basis*----------→

Abbreviations: CD4 = cluster of differentiation 4; CHO = Chinese hamster ovary; eCRF = electronic case report form; ED = exposure day; eDiary = electronic diary; EOS = End of Study; FVIII = coagulation factor VIII; HIV = human immunodeficiency virus; CCI [redacted]; PUP = previously untreated patient; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments:

A: For subjects presenting with a bleeding episode at Screening, it is anticipated that Screening and Visit 1 may occur on the same calendar day, as the diagnosis of hemophilia A is likely to occur within the setting of an emergency.

B: Timing of visits: Visit 1: first rVIII-SingleChain dose in the clinic; monthly visits until 25 EDs with rVIII-SingleChain are achieved; 3-monthly visits thereafter until EOS (ie,75 EDs or early discontinuation). For details, see Section 8.10.2.1.

C: One month is 28 days.

D: Unscheduled visits can be arranged at any time at discretion of investigator or upon request of subject or sponsor. The assessments performed at an unscheduled visit are at discretion of investigator and are to be recorded in the eCRF.

E: Inhibitor-negative PUPs will stay in this study until they have achieved 75 EDs. Additional time in the study may occur for PUPs that develop an inhibitor: CCI [redacted] ii) For inhibitor-positive PUPs who remain in the **main study** who have achieved eradication, the EOS visit will occur when they complete the 12-month post eradication follow-up period in the main study. The maximum duration of the inhibitor treatment period is 24 months starting when the dose is initially adjusted to treat the inhibitor, and if no dose adjustment is performed to treat the inhibitor then the duration of inhibitor treatment will be from the date of inhibitor diagnosis.. In all cases, the EOS Visit should take place at the end of the planned treatment period, if the subject discontinues prematurely, or if the sponsor terminates the study.

F: If Screening and Visit1 do not occur on the same calendar day, inclusion / exclusion criteria will be reviewed again to confirm subject eligibility.

G: If no medical record. Blood sample for gene defect should preferably be taken at Visit 1. If this is not possible, the sample can be taken at any time during the study.

H: Hematology: hemoglobin, hematocrit, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and platelet count
Serum chemistry: sodium, potassium, chloride, blood urea nitrogen, creatinine, gamma-glutamyl transferase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, total protein, glucose, calcium, and urea.

I: If the subject has received any blood components or per investigator discretion considering the subject's medical history.

J: Clinical observation and vital signs measurements (blood pressure, heart rate, and body temperature) should be performed before and at 1, 2, 3, and 6 hours (± 15 minutes) after the first rVIII-SingleChain injection and must be recorded in the eCRF.

K: Blood samples for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies to CHO proteins will be collected at all scheduled visits. For the inhibitor blood samples, subjects should have a 4-day washout from last rVIII-SingleChain injection before blood draw if possible.

L: If the investigator collects blood samples for FVIII levels or inhibitor assessment at any other time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory (see Section 8.6).

M: If not available in subject's medical records, FVIII activity level must be determined at Screening at the local laboratory, and the result must be available before enrollment.

N: Virology retention samples: At Visit 1, the sample must be taken before first rVIII-SingleChain injection. CSL will retain samples for 5 years from EOS.

O: At Visit 1, the investigator will administer first rVIII-SingleChain dose after screening assessments are completed and subject eligibility is confirmed. Subject must be observed at the clinic for at least 6 hours after dosing.

P: Switches to the on-demand regimen and between various prophylaxis regimens are allowed at any time at discretion of the investigator. The eCRF should be updated accordingly for each switch. Subjects **switching to on-demand** should maintain their originally assigned visit schedule.

Q: Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at the next scheduled visit. All unused vials will be returned at the EOS visit.

R: The observation period for adverse event (and serious adverse event) reporting in an individual subject will start at the time of giving written informed consent for participation in the current study and finish with the EOS visit. However, adverse events ongoing at the EOS visit will be followed until resolution or 28 days after the final administration of rVIII-SingleChain during this study, whichever is sooner.

CCI [redacted]

Schedule of Assessments for Arm 2 PUPs WITHOUT Bleeding Episode at Screening and Assigned to ON-DEMAND

Assessments / Visits	Screening (up to 28 days before Visit 1)	Visit 1	Follow-up visits 1 month (± 7 days) after each rVIII-SingleChain dose[A,C,E]	Monthly phone calls (± 7 days) if duration between visits is > 1 month[B,C,E]	EOS[D]
Informed consent / assent	X				
Review of inclusion / exclusion criteria; subject eligibility	X	X[F]			
Demographic information	X				
Medical and surgical history	X				
Hemophilia A history (blood group, gene defect)	X				
Hemophilia A social history and activity level	X	X	X		X
Review of prior medications (previous 28 days)	X				
Blood sample for gene defect[G]	← *At any time*[G] →				
Serum chemistry and hematology[H]	X		X		X
CD4 lymphocyte count (for HIV+ subjects)[I]	X				
Vital signs	X	X[J]	X		X
Physical examination	X		X		X
Body weight and height	X	X	X		X
Inhibitor to FVIII[L]	X[K]		X[U]		X
Antibodies against rVIII-SingleChain and CHO proteins[L]	X[K]		X		X
FVIII activity level	X[M]				
Virology retention sample		X[N]			X
Distribute and train subject on eDiary		X			
Review subject eDiary and collect at EOS visit			X	X	X
Administer rVIII-SingleChain at clinic[O]		**X[O]**	**(X)[P]**		
Investigator assessment for treatment efficacy			X		X
Local tolerability (if rVIII-SingleChain administered at clinic)		X	X		
Assign treatment regimen		X[Q]	X[Q]		
Distribute rVIII-SingleChain		X[R]	X[R]		
Collect used / partially used rVIII-SingleChain vials [S]			X		X
Perform drug accountability			X		X
Record date and details of phone contact				X	

Assessments / Visits	Screening (up to 28 days before Visit 1)	Visit 1	Follow-up visits 1 month (± 7 days) after each rVIII-SingleChain dose[A,C,E]	Monthly phone calls (± 7 days) if duration between visits is > 1 month[B,C,E]	EOS[D]
Confirm that no bleeding episodes have occurred since last visit				X	
Adverse events[T]	←----------*On an ongoing basis*----------→				
Concomitant medications	←----------*On an ongoing basis*----------→				

Abbreviations: CD4 = cluster of differentiation 4; CHO = Chinese hamster ovary; eCRF = electronic case report form; ED = exposure day; eDiary = electronic diary; EOS = End of Study; FVIII = coagulation factor VIII; HIV = human immunodeficiency virus; CCI [redacted]; PUP = previously untreated patient; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments:

A: Timing of visits and distribution of rVIII-SingleChain for home treatment depends on when the subject's bleeding episodes occur. Subjects will return to the site for a follow-up visit 1 month after each rVIII-SingleChain dose. Multiple follow-up visits scheduled to occur within a 10-day time frame may be combined into a single visit (see example in Figure 4). For details, see Section 8.10.2.2.3.

B: Monthly phone calls only for subjects whose next bleeding episode does not occur within 1 month after the preceding visit.

C: One month is 28 days.

D: Inhibitor-negative PUPs will stay in this study until they have achieved 75 EDs; the overall study duration is expected to be up to 6 years. Additional time of up to 3 years in the study may occur for PUPs that develop an inhibitor: CCI [redacted] ii) For inhibitor-positive PUPs who remain in the **main study** who have achieved eradication, the EOS visit will occur when they complete the 12-month post eradication follow-up period in the main study. The maximum duration of the inhibitor treatment period is 24 months starting when the dose is initially adjusted to treat the inhibitor, and if no dose adjustment is performed to treat the inhibitor then the duration of inhibitor treatment will be from the date of inhibitor diagnosis. In all cases, the EOS visit should take place at the end of the planned treatment period, if the subject discontinues prematurely, or if the sponsor terminates the study.

E: Unscheduled visits can be arranged at any time at discretion of investigator or upon request of subject or sponsor. The assessments performed at unscheduled visit are at discretion of investigator and are to be recorded in the eCRF.

F: If Screening and Visit 1 do not occur on the same calendar day, inclusion / exclusion criteria will be reviewed again to confirm subject eligibility.

G: If no medical record. Blood sample for gene defect should preferably be taken at Screening. If this is not possible, the sample can be taken at any time during the study.

H: Hematology: hemoglobin, hematocrit, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and platelet count.
Serum chemistry: sodium, potassium, chloride, blood urea nitrogen, creatinine, gamma-glutamyl transferase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, total protein, glucose, calcium, and urea.

I: If the subject has received any blood components or per investigator discretion considering the subject's medical history.

J: Clinical observation and vital signs measurements (blood pressure, heart rate, and body temperature) should be performed before and at 1, 2, 3, and 6 hours (± 15 minutes) after the first rVIII-SingleChain injection and must be recorded in the eCRF.

K: rVIII-SingleChain administration at Visit 1 does not need to be deferred until the results of inhibitor and antibodies tests are available.

L: Blood samples for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies to CHO proteins will be collected at all scheduled visits. For the inhibitor blood samples, subjects should have a 4-day washout from last rVIII-SingleChain injection before blood draw if possible. If the investigator collects blood samples for FVIII levels or inhibitor assessment at any other time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory (see Section 8.6).

M: If not available in subject's medical records, FVIII activity level must be determined at Screening at the local laboratory, and the result must be available before enrollment.

N: Virology retention samples: The baseline sample must be taken before first rVIII-SingleChain injection. CSL will retain samples for 5 years from EOS.

O: At Visit 1, the investigator will administer first rVIII-SingleChain dose after screening assessments are completed and subject eligibility is confirmed. Subject must be observed at the clinic for at least 6 hours after dosing.

P: During the treatment period, the investigator will administer rVIII-SingleChain dose at the clinic to subjects presenting with a bleeding episodes at these visits.

Q: Switches between on-demand and various prophylaxis regimens are allowed at any time at discretion of the investigator. The eCRF should be updated accordingly for each switch. Subjects **switching from on-demand to prophylaxis** before the start of their monthly visit schedule should immediately switch to monthly visits until 25 EDs and 3-monthly visits thereafter until EOS.

R: rVIII-SingleChain must only be distributed to the subject if the subject / caregiver has received adequate training and if agreeable by the investigator.

S: Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at the next scheduled visit. All unused vials will be returned at the EOS visit.

T: The observation period for adverse event (and serious adverse event) reporting in an individual subject will start at the time of giving written informed consent and finish with the EOS visit. However, adverse events ongoing at the EOS visit will be followed until resolution or 28 days after the final administration of rVIII-SingleChain, whichever is sooner.

CCI [redacted]

Schedule of Assessments for Arm 2 PUPs WITH Bleeding Episode at Screening and Assigned to ON-DEMAND

Assessments / Visits	Screening (up to 28 days before Visit 1)[A]	Visit 1[A]	Follow-up visits 1 month (± 7 days) after each rVIII-SingleChain dose[B,D,F]	Monthly phone calls (± 7 days) if duration between visits is > 1 month[C,D,F]	EOS[E]
Informed consent / assent	X				
Review of inclusion / exclusion criteria; subject eligibility	X	X[G]			
Demographic information	X				
Medical and surgical history	X				
Hemophilia A history (blood group, gene defect)	X				
Hemophilia A social history and activity level	X	X	X		X
Review of prior medications (previous 28 days)	X				
Blood sample for gene defect[H]	← *At any time*[H] →				
Serum chemistry and hematology[I]	X		X		X
CD4 lymphocyte count (for HIV+ subjects)[J]	X				
Vital signs	X	X[K]	X		X
Physical examination	X		X		X
Body weight and height	X	X	X		X
Inhibitor to FVIII[M]	X[L]		X[V]		X
Antibodies against rVIII-SingleChain and CHO proteins[M]	X[L]		X		X
FVIII activity level	X[N]				
Virology retention sample		X[O]			X
Distribute and train subject on eDiary		X			
Review subject eDiary and collect at EOS visit			X	X	X
Administer rVIII-SingleChain at clinic[P]		**X[P]**	**(X)**[Q]		
Investigator assessment for treatment efficacy		X	X		X
Local tolerability (if rVIII-SingleChain administered at clinic)		X	X		
Assign treatment regimen		X[R]	X[R]		
Distribute rVIII-SingleChain		**X**[S]	**X**[S]		
Collect used / partially used vials of rVIII-SingleChain[T]			X		X
Perform drug accountability			X		X
Record date and details of phone contact				X	

Assessments / Visits	Screening (up to 28 days before Visit 1)[A]	Visit 1[A]	Follow-up visits 1 month (± 7 days) after each rVIII-SingleChain dose[B,D,F]	Monthly phone calls (± 7 days) if duration between visits is > 1 month[C,D,F]	EOS[E]
Confirm that no bleeding episodes have occurred since last visit				X	
Adverse events[U]	←----------*On an ongoing basis*----------→				
Concomitant medications	←----------*On an ongoing basis*----------→				

Abbreviations: CD4 = cluster of differentiation 4; CHO = Chinese hamster ovary; eCRF = electronic case report form; ED = exposure day; eDiary = electronic diary; EOS = End of Study; FVIII = coagulation factor FVIII; HIV = human immunodeficiency virus; CCI [redacted]; PUP = previously untreated patient; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments:

A: For subjects presenting with a bleeding episode at Screening, it is anticipated that Screening and Visit 1 may occur on the same calendar day, as the diagnosis of hemophilia A is likely to occur within the setting of an emergency.

B: Timing of visits as well as of distribution of rVIII-SingleChain for home treatment depends on timepoint at which the second bleeding episode occurs. Subjects will return to the site for a follow-up visit 1 month after each rVIII-SingleChain dose. However, multiple follow-up visits scheduled to occur within a 10-day time frame may be combined into a single visit (see example in Figure 4). For details, see Section 8.10.2.3.3.

C: Monthly phone calls only for subjects whose next bleeding episode does not occur within 1 month after the preceding visit.

D: One month is 28 days.

E: Inhibitor-negative PUPs will stay in this study until they have achieved 75 EDs; the overall study duration is expected to be up to 6 years. Additional time of up to 3 years in the study may occur for PUPs that develop an inhibitor: CCI [redacted] ii) For inhibitor-positive PUPs who remain in the **main study** who have achieved eradication, the EOS visit will occur when they complete the 12-month post eradication follow-up period in the main study. The maximum duration of the inhibitor treatment period is 24 months starting when the dose is initially adjusted to treat the inhibitor, and if no dose adjustment is performed to treat the inhibitor then the duration of inhibitor treatment will be from the date of inhibitor diagnosis... In all cases, the EOS visit should take place at the end of the planned treatment period, if the subject discontinues prematurely, or if the sponsor terminates the study.

F: Unscheduled visits can be arranged at any time at discretion of investigator or upon request of subject or sponsor. The assessments performed at unscheduled visit are at discretion of investigator and are to be recorded in the eCRF.

G: If Screening and Visit 1 do not occur on the same calendar day, inclusion / exclusion criteria will be reviewed again to confirm subject eligibility.

H: If no medical record. Blood sample for gene defect should preferably be taken at Screening. If this is not possible, the sample can be taken at any time during the study.

I: Hematology: hemoglobin, hematocrit, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and platelet count.
Serum chemistry: sodium, potassium, chloride, blood urea nitrogen, creatinine, gamma-glutamyl transferase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, total protein, glucose, calcium, and urea.

J: If the subject has received any blood components or per investigator discretion considering the subject's medical history.

K: Clinical observation and vital signs measurements (blood pressure, heart rate, and body temperature) should be performed before and at 1, 2, 3, and 6 hours (± 15 minutes) after the first rVIII-SingleChain injection and must be recorded in the eCRF.

L: rVIII-SingleChain administration at Visit 1 does not need to be deferred until the results of inhibitor and antibodies tests are available.

M: Blood samples for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies to CHO proteins will be collected at all scheduled visits. For the inhibitor blood samples, subjects should have a 4-day washout from last FVIII injection before blood draw if possible. If the investigator collects blood samples for FVIII levels or inhibitor assessment at any other time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory (see Section 8.6).

N: If not available in subject's medical records, FVIII activity level must be determined at Screening at the local laboratory, and the result must be available before enrollment.

O: Virology retention samples: The baseline sample must be taken before first rVIII-SingleChain injection. CSL will retain samples for 5 years from EOS.

P: At Visit 1, the investigator will administer first rVIII-SingleChain dose after screening assessments are completed and subject eligibility is confirmed. Subject must be observed at the clinic for at least 6 hours after dosing.

Q: During the treatment period, the investigator will administer rVIII-SingleChain at the clinic to subjects who present with a bleeding episode at these visits.

R: Switches between on-demand and various prophylaxis regimens are allowed at any time at discretion of the investigator. The eCRF should be updated accordingly for each switch. Subjects switching from on-demand to prophylaxis before their second bleeding episode should immediately switch to the monthly visit schedule for prophylaxis (see Schedule of Assessment for Arm 2 subjects assigned to prophylaxis).

S: rVIII-SingleChain must only be distributed to the subject if the subject / caregiver received adequate training and if agreeable by the investigator.

T: Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at the next scheduled visit. All unused vials will be returned at the EOS visit.

U: The observation period for adverse event (and serious adverse event) reporting in an individual subject will start at the time of giving written informed consent and finish with the EOS visit. However, adverse events ongoing at the EOS visit will be followed until resolution or 28 days after the final administration of rVIII-SingleChain, whichever is sooner.

CCI [redacted]

Schedule of Assessments for Arm 3 (PTPs Not Currently Enrolled in a rVIII-SingleChain Study)

Assessments / Visits	Screening (Up to 28 days before Visit 1	Day 1	Every 3 months[A] (± 7 days)	EOS[B]
Informed consent	X			
Demographic information	X			
Medical and surgical history	X			
Review of inclusion / exclusion criteria; subject eligibility	X			
Hemophilia A history (previous FVIII treatment, blood group, and gene defect)	X			
Hemophilia A social history and activity level	X		X	X
Review of prior medications (previous 28 days)	X			
Blood sample for gene defect, if no medical record[C]		← *At any time during the study*[C] →		
Serum chemistry and hematology[D,E]	X		X	X
CD4 lymphocyte count (for HIV+ subjects)	X			
Vital signs[E]	X	X	X	X
Physical examination[E]	X		X	X
Body weight and height[F]	X	X	X	X
FVIII activity level[G]	X			
Incremental recovery[H]		X	←-----*At specified timepoints*[H] ---→	
Inhibitor to FVIII[I,J]	X		←-----*At specified timepoints*[I] ----→	X
Antibodies against rVIII-SingleChain and CHO proteins[I,J]	X		←-----*At specified timepoints*[I] ----→	X
Virology retention sample[K]		X[K]		X
Distribute and train subject on eDiary		X		
Review subject eDiary and collect at EOS visit			X	X
Assign treatment regimen		X		
Distribute rVIII-SingleChain		X	X[L]	
Record investigator assessment for treatment efficacy			X	X
Assessment of local tolerability (if rVIII-SingleChain administered at clinic)			X	
Collect used and partially used vials of rVIII-SingleChain[M]			X	X
Perform drug accountability			X	X
Adverse events[N]	←----------*On an ongoing basis*----------→			
Concomitant medications	←----------*On an ongoing basis*----------→			

Abbreviations: CD4 = cluster of differentiation 4; CHO = Chinese hamster ovary; eCRF = electronic case report form; ED = exposure day; eDiary = electronic diary; EOS = End of Study; FVIII = coagulation factor FVIII; HIV = human immunodeficiency virus; PTP = previously treated patient; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments:

A: One month is 28 days.

B: The EOS visit should take place at the end of the planned treatment period, if the subject discontinues prematurely, or if the sponsor terminates the study.

C: Blood sample for gene defect should preferably be taken at Day 1. If this is not possible, the sample can be taken at any time during the study.

D: Hematology: hemoglobin, hematocrit, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and platelet count.
Serum chemistry: creatinine, alanine aminotransferase, aspartate aminotransferase, and total bilirubin.

E: Hematology, serum chemistry, physical examinations, and vital signs will be performed during every site visit until the subject reaches 50 EDs, then yearly until EOS.

F: Body weight will be measured for all subjects during each site visit. Height will be measured at each site visit only for subjects < 18 years.

G: If not available in subject's medical records, FVIII activity level must be determined at Screening at the local laboratory, and the result must be available before enrollment.

H: Blood samples will be collected to assess IR and analyzed in the central laboratory. Subjects should have a 4-day washout after last FVIII injection before blood draws if possible. A pre-dose blood sample will be drawn. Subjects will then receive their prophylaxis dose of rVIII-SingleChain injection in the clinic. For on-demand subjects, the usual dose used to treat a bleeding episode or any other dose determined by the investigator will be administered in the clinic. A post-dose blood sample will be drawn 30 to 60 minutes (± 5 minutes) after injection. Incremental recovery will be collected at Day 1, at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs (see Section 8.6.2).

I: Blood samples for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies to CHO proteins will be collected at Screening and at the closest visit after 10 EDs, after 50 EDs, and after 100 EDs. For the inhibitor blood samples, subjects should have a 4-day washout from last FVIII injection before blood draw if possible.

J: If the investigator collects blood samples for FVIII levels or inhibitor assessment at any other time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory (see Section 8.6).

K: Virology retention samples: The Visit 1 sample must be taken before first rVIII-SingleChain injection. CSL will retain samples for 5 years from EOS.

L: rVIII-SingleChain may be dispensed at more frequent schedules if need is determined by the investigator.

M: Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at the next scheduled visit. All unused vials will be returned at the EOS visit.

N: The observation period for adverse event (and serious adverse event) reporting in an individual subject will start at the time of giving written informed consent for participation in the current study and finish with the EOS visit. However, adverse events ongoing at the EOS visit will be followed until resolution or 28 days after the final administration of rVIII-SingleChain during this study, whichever is sooner.

Schedule of Assessments for Surgery Substudy (Arms 1, 2 and 3)

Period	Before surgery	During Surgery	After Surgery
Body weight	X		
Hemoglobin measurement	X		X[A]
Predicted intraoperative estimated blood loss during surgery	X		
Predicted transfusions during surgery	X		
Actual consumption of rVIII-SingleChain	X	X	X
Record surgery type, relationship to hemophilia	X		
Record FVIII plasma activity results and time of collection (if available)[B]	←----------*On an ongoing basis*----------→		
Estimated blood loss during surgery		X	
Additional hemostatic intervention or blood transfusions administered[C]		X	X
Investigator assessment of hemostatic efficacy[D]			X
Presence and extent of surgical wound hematomas			X
Measurement of drainage volume through surgical drains			X
Occurrence of late re-bleeding episodes			X
Inhibitors to FVIII	X[E]		X[F]
Antibodies against rVIII-SingleChain and CHO proteins			X[F]
Adverse events	←----------*On an ongoing basis*----------→		
Concomitant therapy	←----------*On an ongoing basis*----------→		
Date of discharge			X

Abbreviations: CHO = Chinese hamster ovary; eCRF = electronic case report form; FVIII = coagulation factor FVIII; rVIII-SingleChain = single chain recombinant coagulation factor VIII.

Notes to the Schedule of Assessments during Surgery Substudy:

A: Lowest (nadir) hemoglobin measurement after surgery.

B: If the investigator collects a blood sample for FVIII levels at any time during the substudy, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory.

C: Hemostatic interventions or transfusions other than rVIII-SingleChain (eg, other coagulation factors, whole blood, red blood cells, fresh frozen plasma or platelets).

D: Hemostatic efficacy assessment will be determined by the investigator based on a 4-point scale (Section 8.5.2.1).

E: **Arm 2 (PUPs):** Blood sampling for inhibitor testing before surgery is recommended. Inhibitor testing may be performed at the local laboratory, and an additional sample must be sent to the central laboratory.

F: For subjects in all study arms, blood samples will be collected at least 28 days after surgery for analysis at the central laboratory for the presence of inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins.

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List of Abbreviations

Abbreviation	Definition
ABR	Annualized bleeding rate
AE	Adverse event
AESI	Adverse event of special interest
aPCC	Activated prothrombin complex concentrate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AsBR	Annualized spontaneous bleeding rate
BU	Bethesda unit
CHO	Chinese hamster ovary
CI	Confidence interval
COX-2	cyclooxygenase-2
CSL	CSL Behring GmbH
CS	Chromogenic substrate
CVAD	Central venous access device
eCRF	Electronic case report form
ED	Exposure day
eDiary	Electronic diary
EMA	European Medicines Agency
FDA	Food and Drug Administration
FEIBA	Factor eight inhibitor bypassing agent
FVIII	Coagulation factor VIII
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent ethics committee
IMP	Investigational medicinal product
IR	Incremental recovery
IRB	Institutional review board
CCI	CCI
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
PT	Preferred term
PTP	Previously treated patient
PUP	Previously untreated patient
rFVIIa	Activated recombinant factor VII

Abbreviation	Definition
rFVIII	Recombinant factor VIII
rVIII-SingleChain	Single chain recombinant factor VIII
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
US	United States
WFH	World Federation of Hemophilia

1 Introduction

1.1 Background

Hemophilia A is a rare, but serious X-linked recessive bleeding disorder that affects males and is characterized by a deficiency in the plasma protein known as coagulation factor VIII (FVIII). In patients with hemophilia A, primary hemostasis is not affected but fibrin generation is defective because inadequate amounts of thrombin are generated. The patients suffer from spontaneous bleeding episodes, as well as substantially prolonged bleeding upon injury. Rare but life-threatening bleeding may also occur. The patients exhibit a range of clinical severity depending on the extent of residual activity of the deficient factor that can be detected in the circulation of individual patients. The severity of hemophilia A is based upon the activity level (%) of FVIII. The World Federation of Hemophilia (WFH) has defined individuals with < 1% FVIII activity to have severe hemophilia A and characteristically have spontaneous bleeding (predominantly in joints and muscles) that often results in permanent, disabling joint damage. Individuals with 1% to 5% FVIII activity are considered to have moderate hemophilia A. Individuals with 5% to 40% FVIII activity are considered to have mild hemophilia A [WFH, 2012].

The recommended treatment for patients with hemophilia A is to treat or prevent hemorrhage with clotting FVIII replacement therapy. However, some patients develop inhibitors against the exogenous clotting factor, causing these patients to become refractory to replacement therapy with the deficient factor. Inhibitor development occurs in all ethnic types and with both plasma-derived and recombinant FVIII concentrates [DiMichele, 2007]. The actual percentage of patients who develop inhibitors to FVIII fluctuates in the literature with reports of up to 30% of all patients with hemophilia A developing inhibitors [Gouw et al., 2013].

Inhibitors are typically classified as either high- or low-responding, depending on how an individual's immune system is stimulated following repeated exposures to FVIII. High-responding inhibitors can rise quickly to very high levels (Bethesda titer ≥ 5 units) if the immune system reacts briskly or strongly to repeated exposure to FVIII. In high-titer inhibitors without continued exposure (a period of months or years), the titer level could decrease to a low level. Low-responding inhibitors demonstrate a slower or weaker responding immune system and the Bethesda titer will remain low (Bethesda titer < 5 units). However, the characteristics of an inhibitor can change over time. Low-titer inhibitors may also disappear spontaneously within several weeks or months, achieving immune tolerance in patients [DiMichele, 2008].

"The union of Factor VIII with its inhibitor is not associated with allergic reactions. The presence of an inhibitor does not change the typical site, frequency, or severity of bleeding, however the inhibitor makes control of hemorrhages more difficult." [Kasper, 2004].

Inhibitor development usually occurs between a median age of 1.7 and 3.3 years regardless of the replacement factor product used, and occurs less frequently after the age of 5 years. Inhibitors are generally detected between a mean of 9 and 21 exposure days (EDs) with a median of 12 EDs following exposure to factor replacement products. Inhibitor development is most commonly detected within the first 50 EDs, and new inhibitors are rarely detected after 200 treatment days with the replacement factor [DiMichele, 2002].

Previously untreated patients (PUPs) are at particular high risk to develop high- and low-titer inhibitors. In the most recent studies (SIPPET), a 35.4% cumulative incidence of inhibitors in patients with severe hemophilia was reported within the first 50 EDs [Peyvandi et al., 2016]. In patients with inhibitors, particularly high titer, further replacement therapy with FVIII is futile and bleeds must be treated with by passing products [EMA reflection paper on ITI, 2013]. However, bypassing agents are not as effective as FVIII replacement in patients without an inhibitor [Witmer and Young, 2013]. Inhibitor eradication has been achieved with immune tolerance induction (ITI) therapy in a significant proportion of patients (approximately 70% to 85%)[Oldenburg et al., 2014]. Since patients with inhibitors have a higher disease burden and an increased mortality and morbidity compared to those without inhibitors [Witmer and Young, 2013; Walsh et al., 2015], inhibitor eradication by ITI is the gold standard of therapy.

The main goal of ITI is to restore responsiveness to FVIII treatment [Mariani et al., 2003]. ITI treatment regimens utilize ongoing, frequent, uninterrupted exposure to FVIII over a period of a few months to 2 or more years with the goal of inducing antigen-specific tolerance [DiMichele, 2007]. Multiple ITI dosing regimens have been established in different parts of the world without clear evidence of the superiority of one regimen over the other. Low-dose regimens typically utilize substitution of 50 IU/kg 3 times weekly, whereas intermediate- to high-dose regimens employ intense therapies with 100 IU/kg daily or twice daily. While these doses would lead to high FVIII levels in patients without inhibitors and, in single cases, might constitute a risk for thromboembolic events, patients with inhibitors have a marginal incremental recovery (IR) of FVIII, resulting in low or non-detectable FVIII levels in plasma during ITI therapy. To compensate for increasing FVIII activity in successful or partially successful ITI (ie, declining inhibitor titers), some physicians decrease the treatment intensity when the inhibitor titer is found to decrease and the IR of FVIII found to

increase. Regular monitoring of inhibitor titers (once monthly) is recommended, given the potential dynamic changes in the inhibitor titer during ITI therapy. Successful ITI leads to normalization of the IR and FVIII half-life. Based on the results from the International ITI study and other available evidence, ITI treatment guidelines for patients with hemophilia A have been developed [Valentino et al., 2015].

This extension study is being conducted to:

- gain additional safety and efficacy information on the long-term use of single chain recombinant FVIII (rVIII-SingleChain, CSL627) in patients with severe congenital hemophilia A who were enrolled in prior CSL Behring GmbH (CSL)-sponsored clinical studies with rVIII-SingleChain (hereafter referred to as previously treated patients [PTPs]).
- assess the risk of inhibitor formation to FVIII in patients with severe congenital hemophilia A with no prior exposure to any FVIII product (hereafter referred to as PUPs), in accordance with the European Medicines Agency (EMA) guideline on clinical investigation of recombinant and human plasma-derived factor VIII products [EMA, 2011; EMA, 2016].
- investigate the use of rVIII-SingleChain in ITI in PUPs who develop a confirmed inhibitor to rVIII-SingleChain (ITI substudy), supported by recent evidence-based ITI treatment guidelines [Valentino et al., 2015] and the EMA reflection paper on ITI [EMA, 2013].

1.2 Background Information on rVIII-SingleChain

1.2.1 Overview

rVIII-SingleChain, a unique single-chain recombinant FVIII, is under clinical development for the treatment of subjects with severe hemophilia A.

The structure of FVIII has 2 linked protein chains—a heavy and a light chain. Under certain conditions, this structure can dissociate, resulting in the formation of separated or "dissociated" FVIII chains. rVIII-SingleChain uses a strong, covalent bond that connects the light and heavy chains, thereby creating a single-chain recombinant FVIII that is very unlikely to dissociate.

Nonclinical studies have shown that the molecular integrity of rVIII-SingleChain is significantly increased using this single-chain design, resulting in a more stable, homogenous product. In addition, in-vitro studies have shown that rVIII-SingleChain demonstrates a very

strong affinity for von Willebrand factor and a faster and more efficient binding to von Willebrand factor than wild-type FVIII. The FVIII-von Willebrand factor complex plays an important role in the physiological activity and clearance of FVIII and has been shown to have an influence on the presentation of FVIII to the immune system resulting in the potential of reduced inhibitor formation.

1.2.2 Nonclinical Evaluation

In nonclinical studies, CSL tested the activity of rVIII-SingleChain in a tail clip model of hemophilia A in mice. In this model, rVIII-SingleChain was compared to marketed recombinant products, Helixate FS (manufactured by Bayer Healthcare LLC for CSL Behring) and ReFacto (Wyeth Pharmaceuticals, Inc., Philadelphia, PA). Blood loss was measured after dosing the animals with all 3 recombinant products and a plasma-derived FVIII (Humate-P / Haemate-P, CSL Behring). Plasma levels of FVIII were determined by chromogenic substrate (CS) assay, except for the plasma-derived product. All of the products tested showed comparable efficacy in arrest of bleeding in this animal model. Pharmacokinetic (PK) data from studies in cynomolgus monkeys showed a favorable PK profile of rVIII-SingleChain in comparison to the other products, whereupon the area under the concentration curve for 24 hours and clearance of rVIII-SingleChain improved by a factor of up to 1.6 and 1.8, respectively.

The CS assay correlated best with clotting activity from doses of the rVIII-SingleChain and will be used for potency testing. Further details on the outcomes of nonclinical studies are available in the Investigator's Brochure for rVIII-SingleChain.

1.2.3 Previous Clinical Experience

Study CSL627_1001 is a completed 3-part study. Part 1 was a PK comparison of rVIII-SingleChain and octocog alfa (Advate, Baxter Healthcare Corporation, Deerfield, IL, United States [US]). Part 2 continued treatment of subjects from Part 1 with continued dosing of rVIII-SingleChain on an ongoing basis either as prophylaxis or on-demand treatment and evaluated efficacy and safety. Part 3 of the study evaluated the efficacy and safety of continued dosing of rVIII-SingleChain in subjects with severe hemophilia A. Part 3 included a single-dose, repeat PK assessment in a number of subjects. Parts 2 and 3 also included a surgery substudy. Doses and frequency of dosing for subjects undergoing surgery were based upon the subject's PK profile, rVIII-SingleChain PK data, previous FVIII treatment regimen before enrollment, and bleeding phenotype, and were at the investigator's discretion. The dose regimen of rVIII-SingleChain for subjects undergoing surgery was individualized based

on the type of surgery and the clinical status of the subject to maintain a FVIII activity level recommended by the WFH guidelines [WFH, 2012]. Additional information and results from Study CSL627_1001 are available in the Investigator's Brochure.

Study CSL627_3002 is a completed Phase 3 global, multicenter, open-label clinical study of the safety, efficacy and PK with rVIII-SingleChain in 84 pediatric subjects < 12 years of age, with subjects expected to receive at least 50 EDs to rVIII-SingleChain. Subjects were assigned to either an on-demand or prophylaxis treatment regimen, with the dose and frequency of dosing at the discretion of the investigator and based on WFH guidelines [WFH, 2012]. Additional information and results from Study CSL627_3002 are available in the Investigator's Brochure.

1.3 Study Overview

This study is being conducted to gain safety and efficacy information on the long-term use of rVIII-SingleChain in subjects with severe hemophilia A in addition to data from previous lead-in clinical studies. This study also aims to collect information on inhibitor formation in PUPs 0 to < 18 years of age. The EMA guideline entitled "Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products", adopted 21 July 2011, requires the collection of "…additional clinical data to ensure consistency in the long-term between the outcome from pre-authorization clinical studies and from routine use…", necessitating a post-marketing investigation study. With reference to PUPs, the guideline in effect in 2011 states that "approval of the indication in PUPs will be based on a clinical trial in a minimum of 50 PUPs evaluated for efficacy and safety during at least 50 EDs" [EMA, 2011]. Thus, in line with the EMA guideline in effect at time of study planning and implementation of Amendment 3, this extension study was planned to a) fulfill the EMA requirements for a post-marketing investigation to attain 100 EDs in at least 200 PTPs (completed) and b) acquire efficacy and safety data in at least 50 PUPs attaining at least 50 EDs to rVIII-SingleChain. For PTPs directly rolling over from any of the preceding rVIII-SingleChain clinical studies, EDs for rVIII-SingleChain are calculated from the first dose of rVIII-SingleChain, including all EDs to rVIII-SingleChain from any study or substudy.

CCI [redacted]

The EMA guideline has since been updated in 2018 and states "In light of increasing scientific knowledge [Gouw et al., 2013; Calvez et al., 2014; Collins et al., 2014; Peyvandi et

al., 2016], the number of suitable patients especially previously untreated patients (PUPs) to be enrolled in clinical trials is problematic. Hence, the conduct of sufficiently informative clinical trials in PUPs to estimate important characteristics of single products is considered difficult. Therefore the obligation to perform clinical trials in PUPs for marketing authorisation purposes has been deleted [EMA, 2108]." CSL Behring has reached an agreement with the EMA Paediatric Committee through a request for modification of the pediatric investigational plan (PIP) to stop enrolling subjects into Study CSL627_3001 and to continue treatment of the subjects currently enrolled. In addition, to ensure that patients achieve PTP status at the end of participation in Arm 2, all PUPs were to achieve 150 EDs to rVIII-SingleChain. However, based on a recently published report of risk periods for inhibitor development in 1038 PUPs with severe hemophilia A, there is now strong evidence from the largest prospective cohort study of PUPs to show that almost all inhibitors have developed by 75 EDs of treatment [Van den Berg et al., 2019]. CCI [redacted]

The study population will consist of a total of at least 224 subjects with severe hemophilia A (FVIII activity level of < 1%). Of these, at least 200 are PTPs who have participated in a previous CSL-sponsored study with rVIII-SingleChain (ie, Arm 1) or who are not currently enrolled in a rVIII-SingleChain study (Arm 3), and at least 24 are PUPs (ie, Arm 2).

In line with the EMA guideline, Arm 2 of the present study was opened for enrollment after the following information became available:

a) Independent Data Monitoring Committee (IDMC) approval that the data from at least 20 PTPs < 12 years of age with 50 EDs each (including at least 10 PTPs 0 to < 6 years of age and at least 10 PTPs 6 to < 12 years of age) in Study CSL627_3002 does not reveal any safety concern.

b) The rVIII-SingleChain PK in children is adequately characterized from Study CSL627_3002.

In addition, Arm 3 remained closed until the sponsor determined to open it. Arm 3 was opened in order to achieve a total of at least 200 PTPs for 100 EDs in Arms 1 and 3 combined. The sponsor contacted investigators directly when Arm 3 was open for enrollment.

rVIII-SingleChain will be administered intravenously (IV) at an actual dose and on a dosing schedule at the investigator's discretion. This study will be conducted in compliance with this

study protocol, International Conference of Harmonisation (ICH) principles of Good Clinical Practice (GCP), and the applicable regulatory requirement(s) (see Section 13.1).

1.4 Potential Risks and Benefits

The risks include the possibility that subjects treated with rVIII-SingleChain may develop an inhibitor and that antihemophilic factors may therefore be less effective or ineffective in increasing FVIII levels and stopping or preventing bleeding. Treatment with any antihemophilic factor presents a risk of inhibitor formation particularly in PUPs in which the risk of inhibitor formation has been estimated to be up to 35.4% [SIPPET study, Peyvandi et al., 2016; Kasper, 2004]. The development of an inhibitor is associated with significant morbidity and mortality, including a higher rate of bleeding complications, increased disability and a decreased quality of life [Witmer and Young, 2013]. The absolute risk of inhibitor formation with rVIII-SingleChain is currently unknown. In Study CSL627_1001 in 174 PTPs with previous exposure to > 150 EDs to FVIII products, 120 subjects achieved ≥ 50 EDs, of whom 52 subjects had ≥ 100 EDs to rVIII-SingleChain at the end of the study.

For subjects in all study arms, the potential benefits of treatment include stopping and / or preventing bleeding. In addition, rVIII-SingleChain has demonstrated an adequate safety profile for PTPs. The associated benefit-risk assessment of the study is acceptable for subjects enrolled.

Please refer to the Investigator's Brochure for more detailed information.

2 Study Objectives and Endpoints

Overall, the study objectives in all 3 study arms are to gain additional safety information on the incidence of FVIII inhibitors (including in PUPs), frequency of adverse events (AEs), and serious AEs (SAEs) associated with the use of rVIII-SingleChain, and to gain additional information on the efficacy of rVIII-SingleChain in PTPs and PUPs with severe hemophilia A. This study will collect information on subjects undergoing surgery during the study, thereby providing information on the safety and efficacy of rVIII-SingleChain during surgery. CCI

2.1 Primary Objective and Endpoint

2.1.1 Primary Objective

In **Arms 1 and 3 (PTPs)**, the primary objective is to characterize the long-term safety profile of rVIII-SingleChain with respect to inhibitor development in PTPs.

In **Arm 2 (PUPs)**, the primary objectives are:

- To characterize the safety with respect to inhibitor development in PUPs.
- To evaluate the efficacy of on-demand and prophylaxis treatment of rVIII-SingleChain in PUPs.

2.1.2 Primary Endpoints

In **Arms 1 and 3 (PTPs)**, the primary endpoint is the incidence of inhibitor formation to FVIII in at least 200 PTPs with at least 100 EDs of rVIII-SingleChain.

In **Arm 2 (PUPs)**, the primary endpoints are:

- Incidence of high-titer inhibitor formation to FVIII (ie, inhibitor titer of ≥ 5 Bethesda units [BU]/mL) in PUPs with at least 50 EDs of rVIII-SingleChain.
- Treatment success for **major** bleeding episodes, defined as a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale.
- Annualized spontaneous bleeding rate (AsBR) during prophylaxis and on-demand treatment.

2.2 Secondary Objectives and Endpoints

2.2.1 Secondary Objectives

In **Arms 1 and 3 (PTPs)**, the secondary objectives are:

- To characterize the safety profile of rVIII-SingleChain with respect to inhibitor development after 10 EDs and after 50 EDs.
- To characterize the safety profile of rVIII-SingleChain with respect to antibodies against rVIII-SingleChain and antibodies to Chinese hamster ovary (CHO) proteins.
- To collect and evaluate efficacy information on the prophylaxis and treatment of bleeding episodes.
- To assess the hemostatic efficacy of rVIII-SingleChain for PTPs who undergo surgery, using the 4-point efficacy evaluation of surgical treatment scale.

In **Arm 2 (PUPs)**, the secondary objectives are:

- To further characterize the safety profile of rVIII-SingleChain with respect to inhibitor development.
- To characterize the safety profile of rVIII-SingleChain with respect to antibodies against rVIII-SingleChain, and antibodies to CHO proteins.
- To collect and evaluate the number of rVIII-SingleChain injections required for the treatment of bleeding episodes.
- To characterize consumption of rVIII-SingleChain in prophylaxis, on-demand treatment, and surgery.
- To assess the hemostatic efficacy of rVIII-SingleChain for PUPs who undergo surgery, using the 4-point efficacy evaluation of surgical treatment scale.
- To assess the occurrence of clinically significant abnormalities in vital signs after rVIII-SingleChain administration.

2.2.2 Secondary Endpoints

2.2.2.1 Efficacy

In **Arm 1 and Arm 3 (PTPs)**, the secondary efficacy endpoints are:

- Annualized bleeding rate (ABR) (traumatic and non-traumatic) during prophylaxis and on-demand treatment.
- Treatment success for bleeding episodes defined as a rating of "excellent" or "good" on the investigator’s clinical assessment of hemostatic efficacy 4-point scale.

- Percentage of bleeding episodes requiring 1, 2, 3, or > 3 injections of rVIII-SingleChain to achieve hemostasis.
- Mean actual dose per kg per subject per year; consumption of rVIII-SingleChain, expressed as number of injections and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).
- Treatment success for surgery, using the 4-point efficacy evaluation of surgical treatment scale.

In **Arm 2 (PUPs)**, the secondary efficacy endpoints are:

- Treatment success for **non-major** bleeding episodes, defined as a rating of "excellent" or "good" on the investigator's clinical assessment of hemostatic efficacy 4-point scale.
- Percentage of bleeding episodes requiring 1, 2, 3 or > 3 injections of rVIII-SingleChain to achieve hemostasis.
- ABR during prophylaxis and on-demand treatment.
- Mean actual dose per kg per subject per year; consumption of rVIII-SingleChain, expressed as number of injections and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).
- Treatment success for surgery, using the 4-point efficacy evaluation of surgical treatment scale.

2.2.2.2 Safety

In **Arm 1 and Arm 3 (PTPs)**, the secondary saf ety endpoints are:

- Incidence of inhibitor formation to FVIII in at least 200 PTPs after 10 EDs and after 50 EDs with rVIII-SingleChain.
- Percentage of PTPs who develop antibodies against rVIII-SingleChain.
- Percentage of PTPs who develop antibodies to CHO proteins.

In **Arm 2 (PUPs)**, the secondary safety endpoints are:

- Incidence of high-titer inhibitor formation to FVIII (ie, inhibitor titer of ≥ 5 BU/mL) after 10 EDs with rVIII-SingleChain in PUPs.
- Incidence of low-titer inhibitor formation (ie, inhibitor titer of < 5 BU/mL) to FVIII after 10 EDs and after 50 EDs with rVIII-SingleChain in PUPs.
- Incidence of total (low- and high-titer) inhibitor formation to FVIII after 10 EDs and after 50 EDs with rVIII-SingleChain in PUPs.
- Percentage of PUPs who develop antibodies against rVIII-SingleChain.

- Percentage of PUPs who develop antibodies to CHO proteins.
- Percentage of PUPs with clinically significant abnormal values for blood pressure, or heart rate, or body temperature at 1, 2, 3, or 6 hours after the first rVIII-SingleChain injection.
- Percentage of PUPs with treatment-emergent clinically significant abnormal values for blood pressure, heart rate, or body temperature during the course of the study.

2.3 CCI

2.3.1

CCI

2.3.2 CCI

CCI

CCI [redacted]

2.4 Safety Variables Assessed

In all study arms, the following routine safety variables will be assessed:

- Frequency, severity and seriousness of AEs.
- Laboratory values over time, individual subject changes, and clinically significant abnormal values.
- Vital signs values over time, individual subject changes, and clinically significant abnormal values.
- Physical examination.
- Subject / caregiver assessment of local tolerability.

In **Arm 2 and Arm 3 only**, the following will be assessed in addition:

- Investigator assessment of local tolerability (for rVIII-SingleChain injections administered at the clinic).

CCI [redacted]

3 Study Design

3.1 Study Design and Rationale

This multicenter, non-randomized, open-label, multiple-arm phase 3 extension study will continue to investigate the safety and efficacy of rVIII-SingleChain in PTPs with severe hemophilia A as well as in PUPs. This study will evaluate the prophylaxis and on-demand treatment of bleeding episodes in at least 200 PTPs who achieve at least 100 EDs as well as in PUPs who achieve at least 50 EDs. A surgical substudy (open to subjects from all study arms) will investigate the use of rVIII-SingleChain in surgery. CCI [redacted]

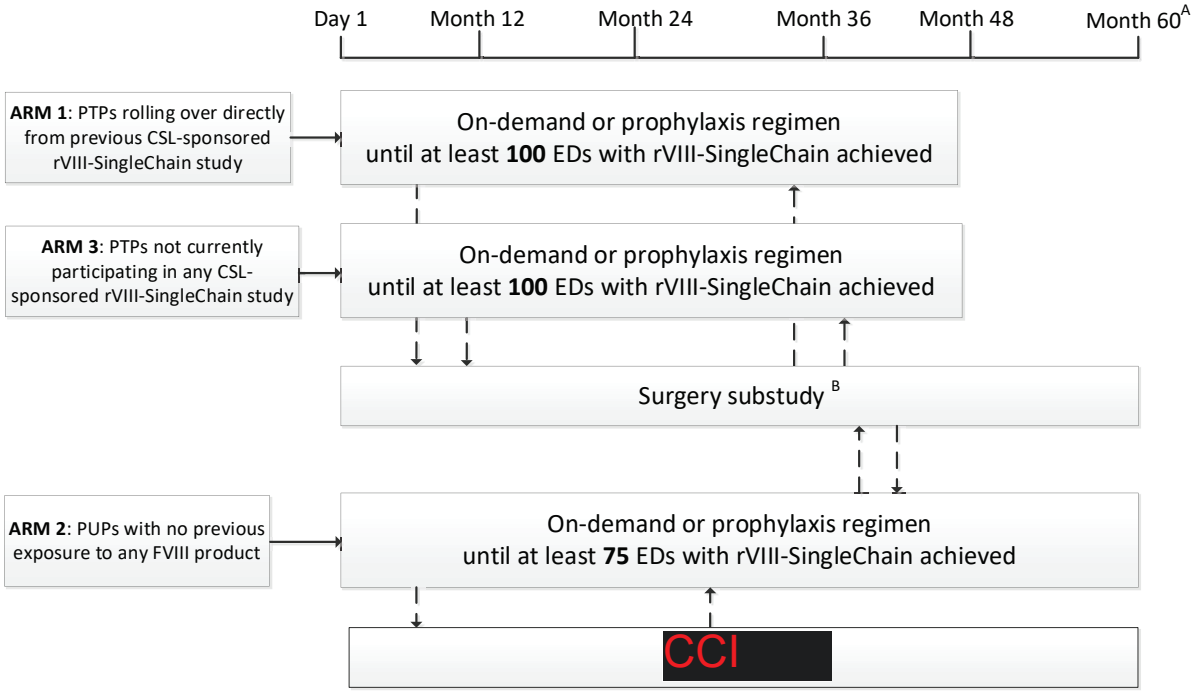
Eligible subjects will be males who have been diagnosed with severe hemophilia A (FVIII activity levels < 1%). Subjects in Arms 1 are PTPs of any age who have participated in a previous CSL-sponsored clinical study with rVIII-SingleChain. Subjects in Arm 2 are PUPs

0 to < 18 years of age who have not participated in any clinical study with rVIII-SingleChain and have no other prior exposure to any FVIII product. Subjects in Arm 3 are PTPs 0 to < 65 years of age previously exposed to FVIII products (which may include rVIII-SingleChain) but who are not currently enrolled in a rVIII-SingleChain study.

Arm 2 PUPs can enter this study either at the time of their first bleeding episode or previously diagnosed with hemophilia A in a non-bleeding state.

This study is designed to collect data on the use of rVIII-SingleChain to assess long-term safety and efficacy beyond the outcome from pre-authorization clinical studies as well as to assess inhibitor development and characterize efficacy and safety of rVIII-SingleChain in PUPs. Inhibitor development is of particular interest for the safety of rVIII-SingleChain.

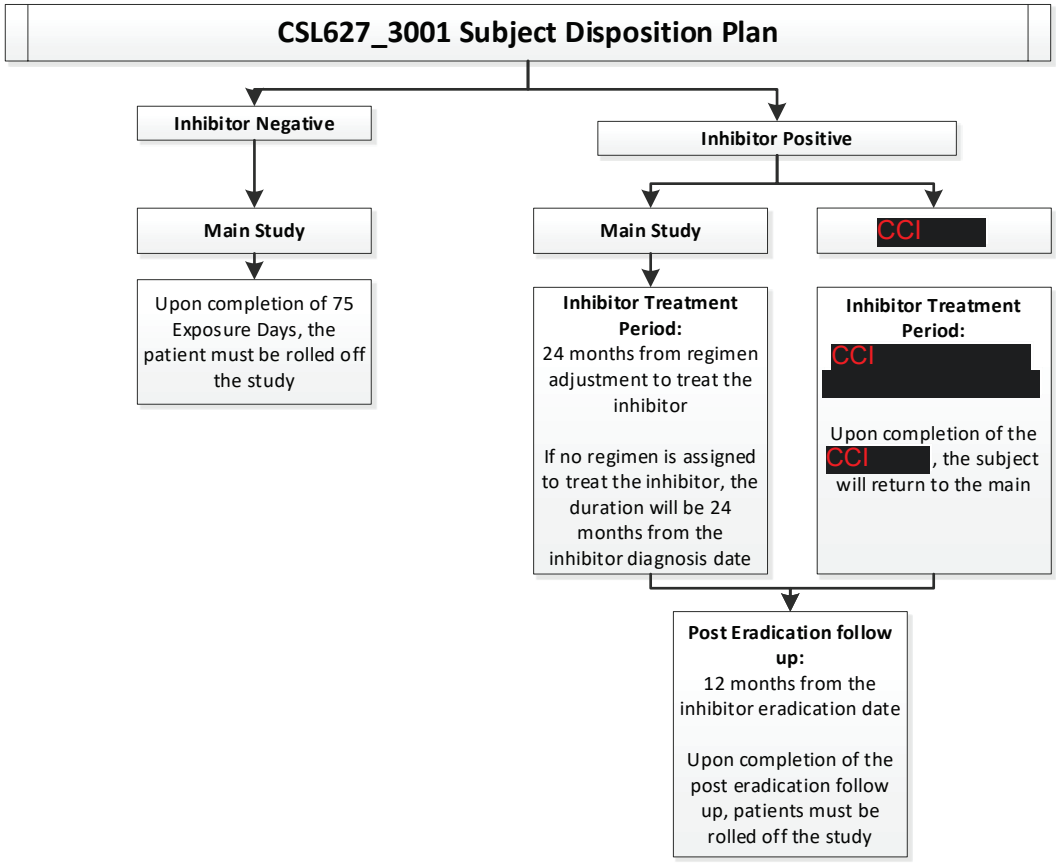
Figure 1: Study Overview of CSL627_3001



Abbreviations: ED = exposure day; PTP = previously treated patient; PUP = previously untreated patient.
A: Estimated duration of study participation to achieve the indicated number of EDs to rVIII-SingleChain.
B: All subjects who participate in Study CSL627_3001 are eligible for the surgery substudy at any time during the study.

CCI

Figure 2: Disposition Plan for PUPs



Abbreviations: CCI ; PUP = previously untreated patient.

3.2 Dose and Dosing Regimen

In all study arms, the investigator will assign subjects to prophylaxis or on-demand treatment regimens.

3.2.1 Prophylaxis Treatment Regimen

In previous studies, most subjects received prophylaxis treatment with an initial range of 15 to 50 IU/kg rVIII-SingleChain every second day or 2 to 3 times per week. In all study arms, the dose prescribed will be based on the subject's weight at the most recent visit. All subjects will be dosed according to the actual potency printed on the vial label and will be treated by IV injection.

- In **Arms 1 and 3 (PTPs)**, the investigator will determine the rVIII-SingleChain dose and dosing schedule for the subject based upon the subject's PK profile, rVIII-SingleChain

PK data, previous FVIII treatment regimen, bleeding phenotype (if available), and taking into consideration the WFH guidelines [WFH, 2012].

- In **Arm 2 (PUPs)**, the investigator will determine the rVIII-SingleChain dose and dosing schedule at their discretion, taking into consideration the WFH guidelines [WFH, 2012], subject's age and other disease characteristics (eg, bleeding phenotype).

3.2.2 Treatment of Bleeding Episodes in Subjects on Prophylaxis and in Subjects On-Demand Treatment Regimens

In the event of a bleeding episode, subjects will be treated at a dose pre-determined by the investigator based on the type and severity of the bleeding episode. All subjects should treat bleeding episodes with rVIII-SingleChain when they occur, regardless of the assigned treatment regimen. The desired FVIII level for the treatment of a bleeding episode (on-demand treatment) is based on the recommendations of the WFH [WFH, 2012].
All subjects will be dosed according to the actual potency printed on the vial label and will be treated by IV injection.

3.2.3 Preventive and Additional Doses

In all study arms, preventive doses of rVIII-SingleChain are allowed. A preventive dose is defined as a dose that is taken prior to an activity or a minor procedure to prevent or minimize a bleeding episode.

In all study arms, additional doses of rVIII-SingleChain are allowed. An additional dose is defined as a dose that is taken beyond the need to control hemostasis and does not contribute to the efficacy evaluation of the bleeding episode. These preventive and additional doses will not contribute to the efficacy evaluation of the bleeding episode but should be captured in the subject's electronic diary (eDiary) or electronic case report form (eCRF), if administered in the hospital. Preventive and additional doses will contribute to EDs and consumption data.

3.2.4 Surgery Doses

In all study arms, the investigator will determine the rVIII-SingleChain dose and treatment schedule for a subject who is scheduled for surgery based on the type of surgery and the clinical status of the subject. The PK data of the study subject or population (if available) will be utilized for calculating the dose regimen of rVIII-SingleChain before, during and after surgery to achieve and maintain the FVIII activity level recommended by the WFH Guidelines [WFH, 2012].

A subject who is required to have surgery during the study will be treated with rVIII-SingleChain before surgery, during surgery, and after surgery, under the supervision of and as prescribed by the treating physician. The subject's normal study routine will be temporarily suspended for the duration of the surgery substudy and the subject will return to the normal study routine after he is determined to be hemostatically stable following surgery as determined by the investigator. Start and end date of the surgical period should be documented in the eCRF.

3.2.5 Change in Treatment Regimens and Dose Modifications

In all study arms, changes in treatment regimens (on-demand or prophylaxis) and dose modifications are allowed and will be permitted at the investigator's discretion. The timing of dose adjustment should be flexible and based on the type of bleeding episode, the location of the bleeding episode, the age of the patient, and bleeding phenotype. The eCRF should be updated accordingly for each change in treatment regimen. Compliance with the assigned dose regimen will be assessed using the criteria detailed in Section 6.2.4.

Special considerations for Arm 2 (PUPs):

- Arm 2 PUPs **switching from on-demand to prophylaxis** during the treatment period should have monthly visits until 25 EDs, followed by visits every 3 months until End of Study.
- Arm 2 PUPs **switching from prophylaxis to on-demand** during the treatment period will have follow-up visits 1 month after each rVIII-SingleChain dose (see Section 8.10.2 for details).

3.2.6 CCI

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3.3 Planned Study Duration

The duration of the study for an individual subject is expected to be approximately 6 years to achieve the following number of EDs with rVIII-SingleChain:

- PTPs in Arm 1 may enroll immediately after participation in previous rVIII-SingleChain studies and continue treatment until achieving at least 100 EDs (including EDs from any previous rVIII-SingleChain study).

- Inhibitor-negative PUPs in Arm 2 will receive treatment in this study until they achieve 75 EDs with rVIII-SingleChain; the overall study duration is expected to be up to 6 years. Additional time of up to 3 years in the study may occur for PUPs that develop an inhibitor:
  - CCI [redacted]
  - For inhibitor-positive PUPs who remain in the **main study** who have achieved eradication, the End of Study Visit will occur when they complete the 12-month post eradication follow-up period in the main study. The maximum duration of the inhibitor treatment period is 24 months starting when the dose is initially adjusted to treat the inhibitor, and if no dose adjustment is performed to treat the inhibitor then the duration of inhibitor treatment will be from the date of inhibitor diagnosis.
- PTPs in Arm 3 will receive treatment in this study until they achieve at least 100 EDs with rVIII-SingleChain (excluding EDs from any previous rVIII-SingleChain study that the subject may have participated in).

In all study arms, subjects will complete the study as outlined in Section 3.1 or until the study is terminated by the sponsor.

3.4 Planned Number of Sites

The study is planned to be conducted at approximately 75 study sites in the world.

3.5 Planned Number of Subjects

This study will enroll a sufficient number of subjects diagnosed with severe hemophilia A to achieve a total of at least 200 PTPs with at least 100 EDs (Arms 1 and 3) and at least 24 PUPs.

Any subject requiring surgery during the course of the study may participate in the surgery substudy, with the exception of inhibitor positive PUPs as the surgical procedure should be covered by a bypassing agent and not by rVIII-SingleChain.

CCI [redacted]

3.6 Study Monitoring Procedures

3.6.1 Data and Safety Monitoring Board (Arm 2 [PUPs] Only)

An IDMC will be established to monitor the evolving risk-benefit profile of rVIII-SingleChain in the PUPs in Arm 2, including data from the surgery and CCI substudies. The IDMC will review the data from Arm 2 periodically as well as on an ad-hoc basis, and their recommendations will pertain to Arm 2 only. At any time after review of data of inhibitors or other relevant safety data, the IDMC can recommend to stop or pause Arm 2 of the study or the substudies.

The IDMC is an independent expert advisory group consisting of 3 medically qualified persons with appropriate expertise in subjects with haemophilia A, in the treatment with FVIII replacement therapy and / or evaluation of AEs and laboratory results relevant for detecting inhibitors or any possible safety issues; in addition, there will be 1 independent statistician.

The IDMC will receive reports on confirmed inhibitor formation (including narratives for SAEs) as well as predefined other safety data at the following timepoints:

- Approximately every 3 months for the first 12 months after the first PUP in Arm 2 received their first rVIII-SingleChain dose.
- Approximately every 6 months thereafter until the End of Study Visit for the last PUP in Arm 2.
- In the event that an inhibitor has been identified, a summary table and subject summary will be sent to the IDMC chair to decide if an ad-hoc meeting is required.

IDMC meetings are scheduled to occur approximately at the following time points:

- When the first PUP in Arm 2 has achieved 25 EDs.
- When the first 5 PUPs in Arm 2 have achieved a minimum of 25 EDs each.
- Every 3 months for the first 12 months and every 6 months thereafter.
- If required: ad-hoc meetings in the event that an inhibitor has been identified.
- If required: ad-hoc meetings in the event of a related SAE or related adverse event of special interest (AESI) in the CCI substudy.

Further details on the composition, responsibilities, meeting schedule, data for review, and recommendations of the IDMC are available in the IDMC Charter.

3.6.2 Other Monitoring Committees

3.6.2.1 CSL Safety Management Team

The purpose of the internal cross-functional Safety Management Team is to ensure a systematic proactive approach to developing and performing safety surveillance and risk management, with the primary goal of minimizing risk to subjects and patients during clinical development and post-authorization.

4 Selection and Withdrawal of Subjects

4.1 Eligibility Criteria

The study population (male subjects with severe hemophilia A) will be selected on the basis of the inclusion and exclusion criteria for Arms 1, 2 and 3 described in the sections below. Subject eligibility should be reviewed and documented by an appropriately medically qualified member of the investigator's study team before subjects are included in the study.

4.1.1 Inclusion Criteria

4.1.1.1 Arm 1 (PTPs)

Subjects meeting all of the following inclusion criteria may be enrolled into Arm 1 of the study:

1. Capable of providing written informed consent and willing and able to adhere to all protocol requirements, or the subject's parent(s) or legally acceptable representative(s) capable of providing written informed consent.
2. Participated in a previous CSL-sponsored rVIII-SingleChain investigational study.

4.1.1.2 Arm 2 (PUPs)

Subjects meeting all of the following inclusion criteria may be enrolled into Arm 2 of the study:

1. Age 0 to < 18 years of age with severe congenital hemophilia A (factor VIII activity < 1%, determined in local laboratory before enrollment or documented in subject's medical record).

2. No prior exposure to any FVIII product (with the exception of short-term use of blood products).
3. Capable of providing written informed consent, or the subject's parent(s) or legally acceptable representative(s) capable of providing written informed consent.
4. The subject as well as their parent(s) or legally acceptable representative(s) willing and able to adhere to all protocol requirements.
5. Investigator believes that the subject is willing and able to adhere to all protocol requirements.
6. Investigator believes that the subject's parent(s) or legally acceptable representative(s) is / are willing and able to adhere to all protocol requirements.

Note: Inclusion criteria for the CCI substudy are defined in Appendix 1.

4.1.1.3 Arm 3 (PTPs)

Subjects meeting all of the following inclusion criteria may be enrolled into Arm 3 of the study:

1. Age 0 to < 65 years.
2. Severe congenital hemophilia A (factor VIII activity < 1%, determined in local laboratory before enrollment or documented in subject's medical record).
3. At least 50 EDs to any FVIII product.
4. Capable of providing written informed consent, or the subject's parent(s) or legally acceptable representative(s) capable of providing written informed consent
5. Investigator believes that the subject is willing and able to adhere to all protocol requirements.
6. Investigator believes that the subject's parent(s) or legally acceptable representative(s) is / are willing and able to adhere to all protocol requirements.

4.1.2 Exclusion Criteria

4.1.2.1 Arm 1 (PTPs)

Subjects meeting any of the following exclusion criteria must not be enrolled into the study:

1. Currently receiving a therapy not permitted during the study, as defined in Section 7.3.
2. Previous participation in the current study.

3. Mental condition rendering the subject (or the subject's legally acceptable representative[s]) unable to understand the nature, scope and possible consequences of the study.
4. Known or suspected hypersensitivity to rVIII-SingleChain or to any excipients of rVIII-SingleChain or CHO proteins.
5. Any issue that, in the opinion of the investigator, would render the subject unsuitable for participation in the study.

4.1.2.2 Arm 2 (PUPs)

Subjects meeting any of the following exclusion criteria must not be enrolled into the study:

1. Any first-order family (eg, siblings) history of FVIII inhibitors.
2. Any known congenital or acquired coagulation disorder other than congenital FVIII deficiency (with the exception of resolved vitamin K deficiency of the newborn).
3. Currently receiving a therapy not permitted during the study, as defined in Section 7.3.
4. Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months before Screening.
5. Known or suspected hypersensitivity to rVIII-SingleChain, to any excipients of rVIII-SingleChain, or CHO proteins.
6. Platelet count < 100,000/µL at Screening.
7. Human immunodeficiency virus (HIV) positive subjects (if the patient has received any blood components or per investigator discretion considering the subject's medical history) with a CD4 count < 200/mm^3 in their medical history or at Screening if available results are older than 1 year (HIV-positive subjects may participate in the study and antiviral therapy is permitted, at the discretion of the investigator).
8. Serum aspartate aminotransferase (AST) or serum alanine aminotransferase (ALT) values > 5 times the upper limit of normal (× ULN) at Screening.
9. Serum creatinine values > 2 × ULN at Screening.
10. Demonstrated or suspected inability (eg, language problem or mental condition) or unwillingness to comply with study procedures or history of noncompliance.
11. Employee at the study site, or spouse /partner or relative of the investigator or sub-investigators.
12. Mental condition rendering the subject (or the subject's legally acceptable representative[s]) unable to understand the nature, scope and possible consequences of the study.

13. Any condition that is likely to interfere with evaluation of the investigational medicinal product (IMP) or satisfactory conduct of the study.
14. Any issue that, in the opinion of the investigator, would render the subject unsuitable for participation in the study.

Note: Exclusion criteria for the [redacted] substudy are defined in Appendix 1.

4.1.2.3 Arm 3 (PTPs)

Subjects meeting any of the following exclusion criteria must not be enrolled into the study:

1. Any history of or current FVIII inhibitors.
2. Any first-order family (eg, siblings) history of FVIII inhibitors.
3. Participation in any other FVIII study other than rVIII-SingleChain in the last 3 months before enrollment.
4. Use of any IMP (including rVIII-SingleChain) within 30 days prior to the first rVIII-SingleChain administration in this study.
5. Administration of any cryoprecipitate, whole blood or plasma within 30 days prior to administration of rVIII-SingleChain.
6. Known or suspected hypersensitivity to rVIII-SingleChain, to any excipients of rVIII-SingleChain, or CHO proteins.
7. Any known congenital or acquired coagulation disorder other than congenital FVIII deficiency.
8. Platelet count < 100,000/µL at Screening.
9. Human immunodeficiency virus (HIV) positive subjects with a CD4 count < 200/mm^3, in their medical history or at Screening if available results are older than 1 year (HIV-positive subjects may participate in the study and antiviral therapy is permitted, at the discretion of the investigator).
10. Subjects currently receiving IV immunomodulating agents such as immunoglobin or chronic systemic corticosteroid treatment.
11. Serum AST or serum ALT values > 5 × ULN at Screening.
12. Serum creatinine values > 2 × ULN at Screening.
13. Evidence of thrombosis, including deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction and arterial embolus within 3 months before Day 1.
14. Experienced life-threatening bleeding episode or had major surgery or an orthopedic surgical procedure during the 3 months prior to Day 1.
15. Demonstrated or suspected inability (eg, language problem or mental condition) or unwillingness to comply with study procedures or history of noncompliance.

16. Employee at the study site, or spouse/partner or relative of the investigator or sub-investigators.
17. Re-entry of subjects previously enrolled or participating in the current study.
18. Mental condition rendering the subject (or the subject's legally acceptable representative[s]) unable to understand the nature, scope and possible consequences of the study.
19. Any condition that is likely to interfere with evaluation of the IMP or satisfactory conduct of the study.
20. Any issue that, in the opinion of the investigator, would render the subject unsuitable for participation in the study.

4.2 Subject Withdrawal

4.2.1 Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or CSL for safety, behavioral or administrative reasons (eg, due to an AE, protocol violation, subject noncompliance, and study termination).

In accordance with ICH principles of GCP, the investigator always has the option to advise a subject to withdraw from the study if the subject's safety or well-being is compromised by his or her further participation in the study. Concern for the interests of the subject must always prevail over the interests of the study.

If a subject is withdrawn from the study or further participation is declined, they will continue to have access to medical care and will be treated as per routine medical practice.

Arm 2 (PUPs):
In addition to the general withdrawal criteria described above, PUPs in Arm 2 **must be withdrawn** from the study if they develop any of the following withdrawal criteria:

- Use of any FVIII product other than rVIII-SingleChain (or its marketed available version) during the study (including if used as rescue medication).
- Subject develops a confirmed high-titer inhibitor (> 5 BU/mL confirmed by repeat testing at the central laboratory) but is not willing to participate in the CCI substudy.
- If the subject in the main study or the CCI substudy has successful inhibitor eradication and experiences inhibitor recurrence (relapse; ie, 2 consecutive inhibitor results ≥ 0.6 BU/mL after eradication).

Further withdrawal criteria specific to the CCI substudy are defined in Appendix 1.

Subjects with a **low-titer** inhibitor may either continue in the main study or can be enrolled into the CCI substudy (see Appendix 1), at the discretion of the investigator after discussion with the sponsor.

4.2.2 Procedures for Handling Withdrawals

If a subject declines further participation or is withdrawn from the study, attempts will be made to complete and document the final assessment. If the subject is withdrawn from the study after receiving rVIII-SingleChain, every effort will be made to ensure that the safety assessments scheduled for the End of Study Visit are completed. The subject may also be asked by the investigator to complete other study assessments.

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, CSL may retain and continue to use any data collected before such withdrawal of consent. Blood specimens may be analyzed to determine safety parameters.

In the event that a subject withdraws from the study, the investigator should record the reason and date of withdrawal in the eCRF and in the subject's medical records.

4.2.3 Replacement Policy

Subjects withdrawn from the study will not be replaced.

5 Study Interventions

5.1 Description of Investigational Product

5.1.1 rVIII-SingleChain (CSL627)

The rVIII-SingleChain drug product is recombinant factor VIII (rFVIII) and is intended for IV injection.

Table 1: Description of rVIII-SingleChain (rFVIII)

Substance number	rVIII-SingleChain (CSL627)
Active substance	FVIII
Trade name	Not applicable
Dosage form	Powder and solvent for solution for injection

Packaging form	Sterile, preservative-free solution for injection containing either 250 IU, 500 IU, 1000 IU, 2000 IU, and 3000 IU per single use vial
Mode of administration	Intravenous injection

Abbreviations: FVIII = coagulation factor VIII.

Each vial of rVIII-SingleChain powder will be reconstituted with 1 vial of sterile water for injection (2.5 mL for 250, 500 or 1000 IU vials; 5.0 mL for 2000 or 3000 IU vials). The label of each rVIII-SingleChain vial will include the actual number of units of FVIII contained within that particular vial. Refer to the Investigator's Brochure for further details.

The study product, rVIII-SingleChain, will be manufactured by CSL Behring GmbH in accordance with Good Manufacturing Practice guidelines and local regulatory requirements.

5.1.2 Comparator Product

Not applicable

5.2 Packaging, Labeling, Supply and Storage

5.2.1 Packaging and Labeling

rVIII-SingleChain will be packaged and labeled according to current ICH Good Manufacturing Practice and GCP guidelines, and national legal requirements.

5.2.2 Supply and Storage

rVIII-SingleChain will be supplied by CSL Behring.

The rVIII-SingleChain study product must be shipped and stored at 2 to 8°C (36 to 46°F) in a closed carton, protected from light. The rVIII-SingleChain study product must not be frozen. The rVIII-SingleChain study product must be stored separately from normal hospital or practice inventories, in a locked facility with access limited to the investigator and authorized personnel. The investigator must ensure that the rVIII-SingleChain study product is dispensed only to subjects enrolled in this study according to this study protocol. Storage and handling instructions of the rVIII-SingleChain study product maintained at the subject's home are described in the subject's IMP handling instructions.

5.3 Accountability and Destruction

The investigator or delegate will confirm receipt of all shipments of the rVIII-SingleChain study product in writing using the receipt form(s) provided by the sponsor or vendor.

All supplies of rVIII-SingleChain must be accounted for throughout the study. At the end of the study, the original Drug Inventory Log, dated and signed by the investigator or delegate (eg, pharmacist), must be retained at the study site as verification of final accountability of rVIII-SingleChain. For Japan sites only, the drug inventory and accountability logs / reports must be dated and signed by the head of the medical institute or the study drug storage manager (if assigned by the head of medical institute).

Records for the delivery of rVIII-SingleChain to the study site, the inventory at the study site, the use by each subject, and the destruction or return of rVIII-SingleChain to CSL must be maintained by the investigator (or delegate).

Any unused or partially used rVIII-SingleChain or empty vials must not be destroyed until the drug accountability documentation has been checked by the study monitor and specific permission for destruction has been given by the sponsor. Any destruction of the rVIII-SingleChain study product must be documented on the form provided for this purpose.

5.4 Other Intervention(s)

Not applicable.

5.5 Rescue Therapy

Other marketed FVIII products should be used as rescue medication only when there is poor / no hemostatic response to rVIII-SingleChain as defined in Section 8.4.1 or when rVIII-SingleChain is not available (eg, emergency use).

The PUPs in Arm 2 using a FVIII product as rescue medication must be withdrawn from the study (see Section 4.2.1). The PTPs in Arms 1 and 3 using a FVIII product as rescue medication do not need to be withdrawn from the study.

The use of bypassing agents (ie, activated recombinant factor VII [rFVIIa] or activated prothrombin complex concentrate [aPCC] / factor eight inhibitor bypassing agent [FEIBA]) is permitted in subjects with inhibitors.

6 Allocation, Dosing and Administration

6.1 Allocation to Treatment

6.1.1 Subject Assignment

After providing written informed consent, a subject identification number will be used to identify the subject for the duration of the study. Subjects in Arm 1 will retain their subject identification number from the preceding rVIII-SingleChain study they participated in.

6.1.2 Randomization Procedures

Not applicable.

6.1.3 Blinding Procedures

Not applicable.

6.2 Dosing and Administration

The investigator (or delegate) will administer or dispense rVIII-SingleChain only to subjects included in this study following the procedures set out in this study protocol.

6.2.1 Prophylaxis Treatment and On-Demand Treatment Regimens

The investigator will assign subjects to either a prophylaxis or on-demand treatment regimen. All subjects should treat bleeding episodes with rVIII-SingleChain when they occur, regardless of the assigned treatment regimen. The rVIII-SingleChain dose and dosing schedule will be determined at the investigator's discretion based upon the criteria detailed in Section 3.2.

Special consideration for PUPs in Arm 2:

1. All PUPs in Arm 2 must receive their first rVIII-SingleChain dose at Visit 1 at the clinic under medical supervision of the investigator (or a delegate experienced in the treatment of hemophilia patients). The subject must remain at the clinic and must be observed for at least 6 hours after this first dose to monitor for signs of injection-related reactions and to perform vital signs assessments. Refer to Sections 8.10.2.1, 8.10.2.2, and 8.10.2.3 for details of the visit schedules and required activities at each visit for Arm 2.

2. If assigned to an on-demand regimen, PUPs in Arm 2 may be provided with rVIII-SingleChain for home treatment after the first dose administered at the site, if the subject / caregiver has received appropriate training for home treatment and if agreeable by the investigator (see Sections 8.10.2.1, 8.10.2.2, and 8.10.2.3).

6.2.2 Preventive or Additional Treatment

In some cases, an additional dose of rVIII-SingleChain beyond what is required to stop a bleeding episode may be taken to maintain hemostasis and this dose will not be counted in the evaluation of efficacy. Preventive and additional doses should be documented in the eCRF. These doses will be counted towards the number of EDs and consumption of rVIII-SingleChain but will not be counted toward the treatment for an on-demand bleeding episode or in the evaluation of efficacy assessment.

6.2.3 Surgery Treatment

Subjects from all study arms may participate in the surgery substudy. The investigator will assign the rVIII-SingleChain dose based on the type of surgery and the subject's clinical status. The subject's previous PK data (if available) can be used to calculate the dose regimen of rVIII-SingleChain before, during, and after surgery to achieve and to maintain a FVIII activity level recommended by the WFH guidelines [WFH, 2012] in Table 2. Any subject required to have surgery during the study should suspend his normally-assigned rVIII-SingleChain treatment, as indicated by the treating physician. Subjects should be treated with rVIII-SingleChain before, during, and after surgery, under the supervision of and as prescribed by the treating physician. Additional injections of rVIII-SingleChain may be needed to maintain the required plasma FVIII activity level.

Table 2: WFH suggested plasma factor level and duration of administration

Type of hemorrhage	Desired FVIII (IU/dL)	Duration (days)
Surgery (major)		
• Pre-operative	80-100	
• Post-operative	60-80	1-3
	40-60	4-6
	30-50	7-14
Surgery (minor)		

• Pre-operative	50-80	
• Post-operative	30-80	1-5, depending on type of procedure

Abbreviations: FVIII = coagulation factor VIII; WFH = World Federation of Hemophilia.
Source: WFH, 2012

6.2.4 CCI

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6.3 Treatment Compliance

Subjects will record in the eDiary the administered dose and time of the dose for any injection of rVIII-SingleChain including prophylaxis, on-demand, prevention, post-surgery treatment of bleeding episodes, and CCI (if applicable). In addition, subjects will bring all of their used and partially used vials of rVIII-SingleChain to the study site at every visit. Unused vials near expiration or with insufficient shelf life to cover time duration until next visit should be returned at next scheduled visit. Treatment compliance will be monitored by counting the number of returned vials and these results will be recorded in the Drug Accountability Log. rVIII-SingleChain use, as reported by the subject in the eDiary, will be reconciled to the returned vials.

To be regarded as compliant to **regimen**, a subject on prophylaxis will have no less than 80% and no more than 120% of the scheduled number of doses during the study period (ie, a subject on 3 times per week who was on study for 10 weeks should have no less than 24 and no more than 36 doses administered for routine prophylaxis).

To be regarded as compliant to **dose**, at least 80% of prophylaxis or bleeding episode treatments doses administered must be within ± 20% of the prescribed dose, ie, a subject assigned to prophylaxis 3 times a week who was on the study for 10 weeks (30 prophylaxis doses) and, in addition treated 10 bleeding episodes (10 bleeding episode treatment doses) was prescribed 50 IU/kg and weighed 80 kg (4000 IU) should have no more than 8 doses outside 3200 to 4800 IU.

7 Contraindications, Permitted Therapies and Prohibited Therapies

7.1 Contraindications and Precautions to Further Dosing

If the subject experiences a hypersensitivity reaction, the study treatment must be stopped immediately by discontinuation of the injection and the medical monitor must be contacted before the administration of additional doses of rVIII-SingleChain.

High-titer inhibitor in Arm 2 (PUPs): If a subject in Arm 2 (PUPs) develops a high-titer inhibitor (confirmed by repeat testing at the central laboratory), no further on-demand or prophylaxis doses of rVIII-SingleChain must be administered to this subject until subject eligibility for the CCI substudy has been confirmed and the subject is enrolled in the CCI substudy (see Appendix 1), unless deemed necessary by the investigator.

Low-titer inhibitor in Arm 2 (PUPs): If a subject in Arm 2 (PUPs) develops a low-titer inhibitor (confirmed by repeat testing at the central laboratory), the following options apply, at the discretion of the investigator after discussion with the sponsor:

- The investigator may decide to enroll the subject into the CCI substudy (see Appendix 1).
- The subject may continue in the main study.

7.2 Permitted Therapies

The following therapy is PERMITTED during the study:

- Blood product transfusion (whole blood, erythrocytes [red blood cells], or platelets) may be used as clinically indicated.
- Plasma and cryoprecipitate should be used only when clinically indicated and not as a substitute for FVIII, unless FVIII is unavailable and as a rescue medication.
- Other marketed FVIII products should be used as rescue medication only when there is poor / no response to rVIII-SingleChain as defined in Sections 8.4.1 and 8.5.2.1 or when rVIII-SingleChain is not available (eg, emergency use, see Section 5.5
  (Note: PUPs from Arm 2 who receive any other FVIII products in this study must be withdrawn [see Section 4.2.1]).
- Bypassing agents are permitted in subjects with inhibitors.

To be used with caution:
With the exception of selective cyclooxygenase-2 inhibitors, nonsteroidal anti-inflammatory drugs are known to have an effect on blood clotting. It is therefore important that the investigator gives careful consideration to the use of nonsteroidal anti-inflammatory drugs in subjects participating in this study. If possible, alternative medication for pain relief, such as paracetamol, which is acetaminophen, should be used.

7.3 Prohibited Therapies

The administration of rVIII-SingleChain is prohibited to any subject not meeting the eligibility criteria for this study, or to any subject not enrolled in this study.

In Arms 1 and 3, the following therapies are NOT PERMITTED during the study:

- Any IMPs other than rVIII-SingleChain.
- Other marketed FVIII products or coagulations factors, except as a rescue medication.

In Arm 2 (PUPs), the following therapies are NOT PERMITTED during the study:

- Any IMPs other than rVIII-SingleChain.
- Other marketed FVIII products.
- Intravenous immunomodulating agents such as immunoglobulin G or > 2 weeks of systemic steroids.

7.4 Dietary and Lifestyle Restrictions

Not applicable.

7.5 Overdose

The effects of any potential overdose with the rVIII-SingleChain study product have not been studied. Any potential overdose should be documented in the eCRF. Section 9.7.1 defines a potential overdose and describes the reporting and documentation procedures.

8 Study Procedures and Visit Schedule

8.1 Clinical Procedures

The clinical procedures that will be conducted during this study related to the evaluation of population demographics and safety are provided in Table 3 and Table 4. Refer to the Laboratory Manual for detailed instructions on how the assessments should be performed.

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Table 3: Clinical procedures: demographics and safety evaluation in Arms 1 and 3 (PTPs)

Assessment	Description
Demographics	Date of birth, age, sex, race and ethnicity
Previous rVIII-SingleChain data	Total rVIII-SingleChain exposure days
Medical and hemophilia A history	Gene defect Blood group Previous FVIII treatment Family history of hemophilia History of inhibitors Co-morbidity or co-medication which would significantly impact blood coagulation or immunoreaction Current / concomitant therapies Hemophilia A history Hemophilia A social history and physical activity level
Physical examination	As per the site's standard procedures
Vital signs	Height and body weight Blood pressure (systolic and diastolic) Heart rate Body temperature
Hematology	Hemoglobin and hematocrit Red blood cell (erythrocyte) count White blood cell (leukocyte) count Platelet count
Serum chemistry	Alanine aminotransferase Total bilirubin Aspartate aminotransferase Creatinine
Serology	Blood samples are to be collected and stored and will be tested if suspicion of viral infection.
Pharmacokinetics	Incremental recovery
Immunogenicity	Blood analyzed for the presence of inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins
FVIII activity level (**Arm 3 only**)	If not available in subject's medical records, FVIII activity level will be determined at the local laboratory at Screening, as per laboratory standard procedures

Assessment	Description
Local tolerability	**Arm 1**: Subject / caregiver assessment (documented in eDiary) **Arm 3**: - Subject / caregiver assessment (documented in eDiary) - Investigator assessment for rVIII-SingleChain injections administered at the clinic

Abbreviations: CHO = Chinese hamster ovary; eDiary = electronic diary; FVIII = coagulation factor VIII; PTP = previously treated patient.

Table 4: Clinical procedures: demographics and safety evaluation in Arm 2 (PUPs)

Assessment	Description
Demographics	Date of birth, age, sex, race and ethnicity
Medical and hemophilia A history	Gene defect Blood group Family history of hemophilia History of inhibitors Co-morbidity or co-medication which would significantly impact blood coagulation or immunoreaction Current / concomitant therapies Hemophilia A history Hemophilia A social history and physical activity level
Physical examination	As per the site's standard procedures. The following body systems must be assessed: general appearance, skin, eyes, ears, nose, throat, heart, lungs, abdomen, neurological system, lymph nodes, spine, and extremities.
Vital signs	Height and body weight Blood pressure (systolic and diastolic) Heart rate Body temperature
Hematology	Hemoglobin and hematocrit Red blood cell (erythrocyte) count White blood cell (leukocyte) count Platelet count

Assessment	Description	
Serum chemistry	Sodium Potassium Chloride Blood urea nitrogen Creatinine Gamma-glutamyl transferase Alkaline phosphatase Alanine aminotransferase	Aspartate aminotransferase Albumin Total bilirubin Total protein Glucose Calcium Urea
Serology	Blood samples are to be collected and stored and will be tested if suspicion of viral infection	
Immunogenicity	Blood analyzed for the presence of inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins	
FVIII activity level	If not available in subject's medical records, FVIII activity level will be determined at the local laboratory at Screening, as per laboratory standard procedures	
Local tolerability	Subject / caregiver assessment (documented in eDiary) Investigator assessment for rVIII-SingleChain injections administered at the clinic	

Abbreviations: CHO = Chinese hamster ovary; eDiary = electronic diary; FVIII = coagulation factor VIII; PTP = previously treated patient; rVIII-SingleChain = single chain recombinant factor VIII.

The timing and frequency of all clinical procedures are described in the Schedules of Assessments.

A positive inhibitor result (local or central) must be repeated and a duplicate sample must be submitted to the central laboratory as soon as possible after receiving the laboratory report to rule out laboratory errors. Refer to the Laboratory Manual for details about the collection, storage, handling and transportation of biological specimens.

8.2 Subject Training

At Day 1 (Arm 1) / Visit 1 (Arms 2 and 3) and all follow-up visits, the investigator or delegate will ensure that the subject has been trained sufficiently to allow for home treatment to occur in the subsequent months of the study, as needed. At this time, the subject will be instructed in the following:

- Correct reconstitution technique for rVIII-SingleChain.
- Correct IV access and administration technique.
- Correct drug storage.

- AE reporting.
- Correct completion of eDiary.

8.3 Treatment Assessments

8.3.1 Subject / Caregiver Treatment Assessments

For treatment received by the subject at home, the subject will report (not limited to) the following information in the eDiary:

- Details of the rVIII-SingleChain administration (eg, total actual IU per injection, number of vials used, and start date and time of injection).
- Type of treatment (routine prophylaxis, on-demand, prevention before activity, additional doses, post-surgery).
- Bleeding episodes, including:
    - Site of the bleeding episode (ie, joint, muscle, mucosal membrane) and specific location.
    - Time of onset of symptoms of bleeding.
    - Reason for use of rVIII-SingleChain and type of the bleeding episode (traumatic or non-traumatic).
    - Date and start time of each injection.
    - Local tolerability assessment.
    - Any concomitant therapy and other hemostatic product usage will be noted in the eDiary and reported to the investigator.

8.3.2 Treatment Assessment Performed at Study Site

Major bleeding episodes are defined as bleeding episodes for which a subject is required to seek treatment at the hemophilia center from the treating physician for any episode that threatens the subject's life or a loss of limb. Examples of major bleeding episodes include intracranial hemorrhage, gastrointestinal bleeding, and severe bleeding. All other bleeding episodes will be classified as non-major unless the investigator assessment notes otherwise.

The following will be recorded in the eCRF following treatment of a bleeding episode by the investigator:

- Reason for use of rVIII-SingleChain and type of the bleeding episode (major or non-major, traumatic or non-traumatic).
- Location of bleeding.

- Time of the start of bleeding.
- Dose of rVIII-SingleChain (IU/kg).
- Number of used vials and total actual IU infused.
- Date and start time of each injection.
- Concomitant therapy and other hemostatic product usage (if any).
- Efficacy evaluation by the investigator.
- AEs.
- Arms 2 and 3 only: investigator assessment of local tolerability if rVIII-SingleChain administered at the clinic.

8.4 Efficacy Evaluation by Investigator

8.4.1 Efficacy Assessment by Investigator for Bleeding Episodes

The investigator will assess the hemostatic efficacy of rVIII-SingleChain after each treated bleeding episode, using the following efficacy evaluation scales for non-major bleeding episodes (Table 5) and major bleeding episodes (Table 6).

Table 5: Efficacy evaluation scale for non-major bleeding episodes by investigator

Category	Description
Excellent	Definite pain relief and / or improvement in signs of bleeding (ie, swelling, tenderness, and / or increased range of motion in the case of musculoskeletal hemorrhage) within approximately 8 hours after the first rVIII-SingleChain injection.
Good	Definite pain relief and / or improvement in signs of bleeding at approximately 8 hours after the first rVIII-SingleChain injection, but requires 2 injections for complete resolution.
Moderate	Probable or slight beneficial effect within approximately 8 hours after the first rVIII-SingleChain injection; requires more than 2 injections for complete resolution.
Poor / No response	No improvement at all or condition worsens (ie, signs of bleeding) after the first rVIII-SingleChain injection and additional hemostatic intervention is required with another FVIII product, cryoprecipitate, or plasma for complete resolution.

Abbreviations: FVIII = coagulation factor VIII; rVIII-SingleChain = single chain recombinant factor VIII.

Table 6: Efficacy evaluation scale for major bleeding episodes by investigator

Category	Description
Excellent	Hemostasis clinically not significantly different from (eg, achieved hemostasis comparable to that expected for a similar bleeding episode in a non-factor deficient patient) and estimated blood loss is not more than 20% higher than the estimated predicted blood loss for the type of injury or problem
Good	Normal or mildly abnormal hemostasis in terms of quantity and / or quality (eg, Slight oozing, prolonged time to hemostasis with somewhat increased bleeding compared to a non-factor deficient patient) or estimated blood loss is greater than 20% but less than or equal to 30% higher than the estimated predicted blood loss for this type of injury or problem
Moderate	Moderately abnormal hemostasis in terms of quantity and / or quality (eg, moderate hemorrhage that is difficult to control) with estimated blood loss greater than what is defined as Good
Poor / No response	Severely abnormal hemostasis in terms of quantity and / or quality (eg, severe hemorrhage that is difficult to control) and / or additional hemostatic intervention required with other FVIII product, cryoprecipitate, or plasma more than expected for the type of injury or problem

Abbreviations: FVIII = coagulation factor VIII.

8.5 Treatment for Surgery

Subjects undergoing surgery may be treated with rVIII-SingleChain (see Section 3.2.4 and Section 6.2.3 for surgery dose and surgery treatment). All subjects undergoing surgery may enroll in the surgery substudy. Subjects who enroll in the surgery substudy will return to the main study at the end of participation, as outlined in Section 8.5.3. Doses of rVIII-SingleChain administered before and after surgery will be documented.

All invasive procedures other than surgery will not be included in the surgery substudy. Subjects undergoing an invasive procedure other than surgery may be treated with rVIII-SingleChain. However, invasive procedures other than surgery are considered medical events of special interest and should be documented in the eCRF.

If a subject has an emergency surgery and rVIII-SingleChain is not available, the subject may substitute rVIII-SingleChain with a marketed FVIII product during surgery; the surgery will

not be included in the surgery substudy. Subjects in **Arms 1 and 3 (PTPs)** may return to the study after the emergency surgery at the discretion of the investigator and sponsor. If a subject has a planned surgery and uses any FVIII product other than rVIII-SingleChain, the subject must be withdrawn from the study.

Subjects in **Arm 2 (PUPs)** must be withdrawn from the study if they receive any FVIII product other than rVIII-SingleChain, irrespective of the type of surgery (emergency or planned) for which that FVIII product was administered. Inhibitor positive patients who require surgery are not eligible for the surgery substudy, as the surgical procedure should be covered by bypassing agent.

8.5.1 Surgery Substudy Clinical Procedures

The timing and frequency of clinical procedures before, during and after surgery are presented in the Schedule of Assessments for Surgery Substudy. The surgical substudy period starts when the first surgery rVIII-SingleChain dose is administered and ends when the subject returns to the scheduled dosing regimen in the main study.

If the investigator collects a blood sample for FVIII activity level or inhibitor assessment at any time during the substudy, the local laboratory results (and time of specimen collection) must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory.

8.5.1.1 Before Surgery

The following procedures will be performed before surgery:

- Obtain body weight.
- Obtain blood sample for hemoglobin measurement (to be assessed by local laboratory).
- **Arm 2 (PUPs) only:** Obtain blood sample for inhibitor testing (recommended). If determined at the local laboratory, the results and time of specimen collection must be recorded in the eCRF. A duplicate sample must be submitted for analysis to the central laboratory.

Before surgery, the surgeon (or physician performing the procedure) will perform the following estimates:

- Predicted intraoperative blood loss (average and maximum): the predicted blood loss is the amount of blood loss predicted by the surgical team (anesthesiologist and / or surgeon) prior to the start of surgery for a patient without hemophilia undergoing the same surgery.

- Predicted transfusions during surgery based on a patient without hemophilia undergoing the same surgical procedure.

The investigator will document the following information before surgery:

- Actual rVIII-SingleChain consumption before surgery (including dose regimen, and number of injections per day).
- Type of surgery (emergency or non-emergency).
- Relationship to hemophilia (related or non-related).
- Local laboratory results of FVIII activity level (and time of specimen collection), if performed (a duplicate sample must be submitted to the central laboratory).
- AEs.
- Concomitant medications.

8.5.1.2 During Surgery

The investigator will document the following information based on data collected during surgery:

- Estimated blood loss as assessed by the surgical team (anesthesiologist and / or surgeon) performing the procedure (including both blood loss expected as integral to the procedure and unexpected blood loss due to unforeseen surgical complications).
- Additional hemostatic interventions and / or transfusions required (including blood products and other coagulation factor[s]) during surgery.
- Actual rVIII-SingleChain consumption during surgery (including dose regimen, and number of injections per day).
- Local laboratory results of FVIII activity level (and time of specimen collection), if performed (a duplicate sample must be submitted to the central laboratory).
- AEs.
- Concomitant medications.

8.5.1.3 After Surgery

The following information will be obtained after surgery:

- Investigator assessment of hemostatic efficacy: The investigator will assess surgery hemostatic efficacy of rVIII-SingleChain by using a 4-point surgical treatment rating scale of “excellent”, “good”, “moderate”, or “poor / no response” (see Table 7 in Section 8.5.2.1).
- rVIII-SingleChain consumption (including dose regimen, and number of injections per day).

- Additional hemostatic intervention or blood transfusions administered.
- Presence and extent of any surgical wound hematomas noting whether they require surgical evacuation.
- Measurement of drainage volume through surgical drains.
- Occurrence of late re-bleeding episodes.
- Lowest (nadir) postoperative hemoglobin level.
- Local laboratory results of FVIII activity level (and time of specimen collection), if performed (a duplicate sample must be submitted to the central laboratory).
- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- AEs.
- Concomitant therapies.
- Record date of discharge.

28-day follow-up laboratory inhibitor sample

- Collect a blood sample at least 28 days after surgery to test for inhibitors to FVIII, antibodies to rVIII-SingleChain, and antibodies to CHO proteins.

8.5.2 Surgery Substudy Assessments

8.5.2.1 Efficacy Assessment by Investigator for Surgery

The investigator will assess the efficacy of rVIII-SingleChain by using a 4-point surgical treatment rating scale of “excellent”, “good”, “moderate”, or “poor / no response” after the end of surgery and record results in the eCRF (see Table 7).

Table 7: Investigator assessment of hemostatic efficacy of rVIII-SingleChain for surgery substudy

Category	Description
Excellent	Hemostasis clinically not significantly different from normal (eg, achieved hemostasis comparable to that expected during similar surgery in a non-factor deficient patient) 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 (eg, slight oozing, prolonged time to hemostasis with somewhat increased bleeding compared to a non-factor deficient patient in the absence of other hemostatic intervention) or estimated blood loss is greater than 20% but less than or equal to 30% higher than the predicted blood loss for intended surgery.
Moderate	Moderately abnormal hemostasis in terms of quantity and / or quality (eg, moderate hemorrhage that is difficult to control) with estimated blood loss greater than what is defined as Good.
Poor / No response	Severely abnormal hemostasis in terms of quantity and / or quality (eg, severe hemorrhage that is difficult to control) and / or additional hemostatic intervention required with another FVIII product, cryoprecipitate, or plasma for complete resolution.

Abbreviations: FVIII = coagulation factor VIII; rVIII-SingleChain = single chain recombinant factor VIII.

8.5.3 Return to Treatment Regimen Following Surgery

Subjects will be treated with rVIII-SingleChain during the recovery period as prescribed by the treating physician.

Following surgery, once the subject is determined to be hemostatically stable and receives their last prescribed post-operative dose, the subject should resume their treatment regimen with rVIII-SingleChain, as determined by the investigator. This is the end of the surgery substudy.

8.6 Blood Samples for Inhibitors, Antibodies, Incremental Recovery and FVIII Activity Level

If the investigator collects a blood sample for inhibitor assessment at any time during the study, the local laboratory results must be recorded in the eCRF and a duplicate sample must be submitted for analysis to the central laboratory. A positive inhibitor result (local or central) must be repeated and a duplicate submitted to the central laboratory as soon as possible after receiving the laboratory report to rule out laboratory errors.

If the investigator collects a blood sample for FVIII activity levels at any time during the study, a duplicate sample must be sent to the central laboratory.

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8.6.1 Blood Samples for Inhibitors and Antibodies

The presence of inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins will be analyzed by the central laboratory. For the inhibitor blood samples, subjects should have a 4-day washout from last FVIII infusion before blood draw if possible.

1. In **Arms 1 and 3 (PTPs)**, blood samples for inhibitors to FVIII, antibodies to rVIII-SingleChain, and antibodies to CHO proteins will be collected at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs as outlined in Table 8.
2. In **Arm 2 (PUPs)**, blood samples for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies to CHO proteins will be collected at all scheduled visits.

Blood samples drawn for inhibitor assessment should be sent to the central laboratory for testing with the Nijmegen assay. A subject will be considered to have developed an inhibitor if the titer result is ≥ 0.6 BU/mL and verified by repeat testing. FVIII inhibitors will be further categorized as low-titer (≤ 5 BU/mL) and high-titer (> 5 BU/mL). If an inhibitor is suspected, all inhibitor testing must be expedited and a repeat sample obtained as soon as possible to confirm results.

Details regarding sample collection, handling, deep freezing of samples and shipment to the central laboratory are given in the Laboratory Manual in the Investigator Site File.

If an inhibitor is suspected (eg, lack of efficacy of IMP), additional visits may be scheduled for inhibitor assessment. If a subject undergoes surgery, blood samples will be collected at least 28 days after surgery to test for inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins in all study arms. In addition, a pre-surgery blood sample for inhibitor testing is recommended for PUPs in Arm 2.

8.6.2 Blood Samples for Incremental Recovery

In Arms 1 and 3 (PTPs), IR will be collected at Day 1 and at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs, as outlined in Table 8. Incremental recovery ([IU/mL] / [IU/kg]) is defined as FVIII activity (IU/mL) obtained 30 to 60 minutes following injection, per dose of (IU/kg) injection. Incremental recovery values will be baseline-corrected for pre-injection plasma FVIII activity.

If possible, subjects should have a 4-day washout from their last FVIII infusion before the IR samples are drawn. A pre-dose blood sample will be drawn. Subjects will then receive their prophylaxis dose of rVIII-SingleChain injection. For on-demand subjects, the usual dose used to treat a bleeding episode may be administered or any other dose determined by the investigator. A post-dose blood sample will be drawn 30 to 60 minutes (± 5 minutes) after the injection. The exact times of blood draws and the actual dose (IU/kg actual potency) administered will be recorded. Blood sample collection timepoints are presented in Table 8.

Table 8: Blood sample collection timepoints for inhibitor and incremental recovery assessment (Arms 1 and 3 [PTPs])

	Day 1	Exposure days of rVIII-SingleChain [A]		
		10 EDs	50 EDs	100 EDs
Inhibitor [B]	From previous End of Study Visit	X	X	X
Incremental Recovery [C]	X	X	X	X

Abbreviations: CHO = Chinese hamster ovary; ED = exposure day; PTP = previously treated patient; rVIII = recombinant factor FVIII.

From "Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products" (EMA, 2011)

A: For Arm 1, exposure days are cumulative from previous lead-in studies (calculated from the first dose of rVIII-SingleChain in any study). For Arm 3, exposure days will be calculated from the first dose of rVIII-SingleChain in the present study only.

B: After 4-day washout period (if possible), inhibitor assessment will include inhibitors to FVIII, antibodies against rVIII-SingleChain, and antibodies against CHO proteins.

C: After 4-day washout period (if possible), blood samples will be drawn: 1) prior to injection of rVIII-SingleChain, and 2) at 30 to 60 minutes (± 5 minutes) following injection. The exact times of blood draws post-injection will be recorded.

CCI [redacted]

8.6.3 Blood Samples for FVIII Activity Level

In Arms 2 and 3, blood samples for FVIII activity level will be obtained at Screening if FVIII activity level is not available in the subject's medical records. If FVIII activity level is determined at Screening, it must be determined at the local laboratory and the result must be available before enrollment.

8.7 Retention of Samples

In Arms 2 and 3, blood samples will be obtained at Visit 1 before the first dose of rVIII-SingleChain and at the End of Study Visit and retained for viral safety testing, if needed. For Arm 1, no additional Day 1 virology retention sample will be taken, but the sample from the End of Study Visit in the preceding study (ie, either CSL627_1001 or CSL627_3002) will be used as the Day 1 sample in the current study.

Retention samples will be stored by CSL for 5 years from the end of the study and will be analyzed only if there is a suspicion of a viral infection.

8.8 Prior and Concomitant Therapies

All drugs taken by a subject within 28 days prior to enrollment into the study are regarded as prior therapy and must be documented as such in the eCRF.

All drugs currently being taken by a subject at enrollment into the study, and which continue to be taken in addition to rVIII-SingleChain during the study, are regarded as concomitant therapy and must be documented as such in the eCRF.

Subjects should not be enrolled into the study if they receive any prohibited concomitant therapy or any concomitant therapy in a prohibited dosage that cannot be discontinued or reduced to a permitted level. Any IMPs other than rVIII-SingleChain are prohibited.

8.9 Assessment of Local Tolerability

8.9.1 Subject / Caregiver Assessment of Local Tolerability

In all study arms, local tolerability at the injection site will be assessed by the subject or the subject's caregiver after each rVIII-SingleChain injection administered by the subject or caregiver. Subjects (if capable) or the subject's caregiver will record in the subject diary a general judgment on the overall perception of the local reactions approximately 30 minutes after the end of the injection on a 5-point scale of none (0), very slight (1), slight (2), moderate (3), and severe (4). In addition, subjects will have the ability to record information in the diary regarding side effects.

8.9.2 Investigator Assessment of Local Tolerability (Arm 2 and Arm 3 Only)

In PUPs in Arm 2 and PTPs in Arm 3, the Investigator will perform an assessment of local tolerability at 30 ±10 minutes after each rVIII-SingleChain injection administered at the clinic.

If several injection sites are used, each site will be judged but only the site with the strongest reaction will be recorded, according to the following scales:

Erythema

Table 9: Erythema Rating Scale

Description	Score
None	0
Very slight (barely perceptible)	1
Well-defined	2
Moderate to severe	3
Severe (beet redness) to slight eschar formations (injuries in depth)	4

Edema or Induration

The size of any edema or induration will be determined by measuring the smallest and largest diameters.

Itching, Local Pain, or Local Heat

The severity of any local reactions of itching, local pain, or local heat will be assessed by questioning the subject based on the following classification: none (0), very slight (1), slight (2), moderate (3), and severe (4).

8.10 Visit Schedules

In all study arms, all subjects must provide written informed consent before any study-specific assessments or procedures are performed. Written informed consent is not required for assessments or procedures performed according to standard of care (eg, for diagnosis or treatment); results from such assessments may be used in the determination of study eligibility.

Written informed parental or guardian consent and assent of minors (if the subject is capable of providing assent) must be obtained before a subject can start any of the procedures at Screening (Arms 2 and 3) / Day 1 (Arm 1). The procedure(s) for obtaining written informed consent and assent of minor (if the subject is capable of providing assent) are described in Section 13.3. Standard of care and local laboratory procedures performed before the signing of informed consent may be used if they occur before Screening / Day 1.

The detailed visit schedules for all study arms are presented in the subsections specified below:

1. **Arm 1 (PTPs)**: Section 8.10.1
2. **Arm 2 (PUPs):** Section 8.10.2
3. **Arm 3 (PTPs)**: Section 8.10.3

8.10.1 Visit schedule for Arm 1 (PTPs)

8.10.1.1 Day 1 Visit

Subjects who have participated in a previous CSL-sponsored rVIII-SingleChain study may enroll directly into Arm 1 of Study CSL627_3001. Data from the End of Study Visit from lead-in studies will be used as Day 1 parameters for this study. The following procedures will be conducted and documented at Day 1:

- Obtain written informed consent and assent of minors.
- Review of inclusion / exclusion criteria for Arm 1.
- Obtain blood sample for gene defect, if not previously obtained or gene defect not previously documented in medical records (Note: This sample should preferably be taken on Day 1. If this is not possible, the sample can be taken at any time during the study).
- Obtain blood sample for CD4 lymphocyte count (for HIV+ subjects).
- Obtain blood sample for IR.
- Record weight and height.
- Distribute eDiary and train subject on correct use.
- Assign treatment regimen (on-demand or prophylaxis).
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.

The following procedures will be conducted and documented at Day 1 if not performed and documented at the previous rVIII-SingleChain study's End of Study Visit:

- Demographic information.
- Medical and surgical history.
- Hemophilia A history (previous FVIII treatment, blood group, and gene defect).
- Hemophilia A social history and activity level.
- Serum chemistry and hematology.
- Vital signs.
- Physical examination.
- Inhibitors to FVIII.

- Antibodies against rVIII-SingleChain.
- Antibodies against CHO proteins.
- Virology retention sample.
- AEs.
- Concomitant medications.

Subjects who complete all of these assessments and who fulfill the eligibility criteria for Arm 1 will be enrolled into the study. If the subject is not eligible for the study, the primary reason for screen failure must be entered in the eCRF.

8.10.1.2 Treatment Period

Subjects in Arm 1 will return to the study site every 3 months (± 7 days) for assessment. At each visit, the following procedures will be conducted and documented:

- Hemophilia A social history and activity level.
- Serum chemistry and hematology (until 50 EDs, then yearly).
- Vital signs (until 50 EDs, then yearly).
- Physical examination (until 50 EDs, then yearly).
- Weight.
- Height only for subjects < 18 years old.
- Incremental recovery (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Inhibitors to FVIII (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Antibodies against rVIII-SingleChain (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Antibodies against CHO proteins (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Review of subject eDiary.
- Distribution of rVIII-SingleChain.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Drug accountability.
- AEs.
- Concomitant medications.

For the procedures at the End of Study Visit, see Section 8.10.6.1.

8.10.2 Visit Schedule for Arm 2 (PUPs)

Summary of visit schedule for PUPs assigned to PROPHYLAXIS:

1. All PUPs assigned to prophylaxis receive their first rVIII-SingleChain dose in the clinic at Visit 1, either as a treatment dose for a bleeding episode, or as their first prophylactic dose under medical supervision.
2. At Visit 1, subjects will be provided with rVIII-SingleChain for prophylaxis therapy at home, if agreeable by the investigator.
3. After Visit 1, subjects will have monthly follow-up visits. Monthly visits continue until the subject reaches 25 EDs.
4. Once 25 EDs are reached, subjects will have 3-monthly follow-up visits until End of Study.

Summary of visit schedule for PUPs assigned to ON-DEMAND:

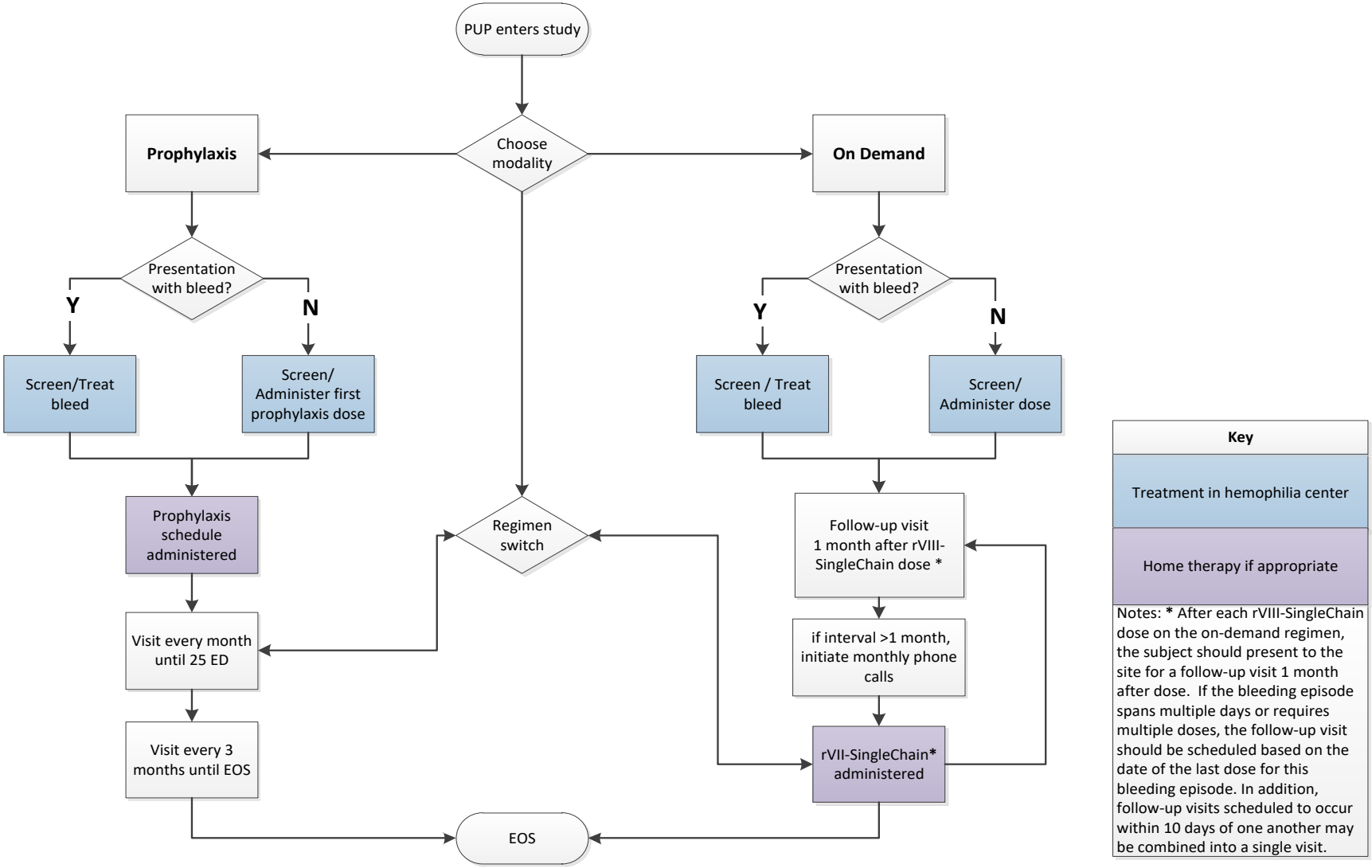
- All PUPs assigned to on-demand will receive their first rVIII-SingleChain dose in the clinic at Visit 1, either as a treatment dose for their first bleeding episode, or as their first exposure under medical supervision.
- Thereafter, the subject / caregiver can treat bleeding episodes with rVIII-SingleChain themselves, if agreeable by the investigator.
- Follow-up visits will occur 1 month after each rVIII-SingleChain dose. The following should be taken into consideration for scheduling these follow-up visits:
    - If the subject has a bleeding episode that spans multiple days or requires multiple doses, the follow-up visit should be scheduled based on the date of the last rVIII-SingleChain dose given for this bleeding episode.
    - If the subject has multiple rVIII-SingleChain doses within a 10-day timeframe, then the associated follow-up visits can be combined into a single visit if they occur ≤ 10 calendar days apart. In contrast, if the doses and the resulting follow-up visits occur at > 10 calendar days apart, separate follow-up visits must be performed (see example in Figure 4).
- If the interval between follow-up visits is > 1 month, monthly phone calls will be performed until the subject returns to the clinic for the next follow-up visit. These phone calls are mainly intended to collect information on bleeding status and AEs.

The timing of visits depends on the treatment assignment (ie, prophylaxis or on-demand) as well as on the clinical presentation at Screening for subjects assigned to an on-demand

regimen (ie, without or with a bleeding episode). This results in 3 different visit schedule options detailed in the following subsections and depicted in the algorithm in Figure 3:

1. PUPs with or without bleeding episode at Screening and assigned to PROPHYLAXIS: Section 8.10.2.1
2. PUPs WITHOUT bleeding episode at Screening and assigned to ON-DEMAND: Section 8.10.2.2
3. PUPs WITH bleeding episode at Screening and assigned to ON-DEMAND: Section 8.10.2.3

Figure 3: Algorithm for treatment assignment and visit structure in Arm 2 PUPs



Abbreviations: ED = exposure day; EOS = End of Study; INH = inhibitor; N = no; PUP = previously untreated patient; Y = yes.

Figure 4: Example of visit structure for PUPs assigned to on-demand treatment

Follow-up visits scheduled to occur within 10 days of one another may be combined into one follow-up visit. Combinations are at the Investigator's discretion. Below is an example of possible combinations of follow-up visits.

- Visit 2 is the follow-up visit for the first rVIII-SingleChain dose at Screening/Visit 1
- Visit 3 is the combined follow-up visit for rVIII-SingleChain doses 2 and 3.
- Visit 4 is the combined follow-up visit for rVIII-SingleChain doses 4 and 5.
- Due to the timing of rVIII-SingleChain doses, additional combinations of follow-up visits are possible.

Abbreviations: PUP = previously untreated patient

8.10.2.1 Visit Schedule for Arm 2 PUPs With or Without Bleeding Episode at Screening and Assigned to PROPHYLAXIS

8.10.2.1.1 Screening

For subjects presenting with a bleeding episode at Screening, it is anticipated that the Screening and Visit 1 assessments (ie, administration of first dose of rVIII-SingleChain and relevant efficacy assessments) may occur on the same calendar day due to the emergency nature of such presentation. The following procedures will be conducted and documented at Screening (within 28 days before Visit 1):

- Obtain written informed consent and assent of minors.
- Review of inclusion / exclusion criteria for Arm 2.
- Demographic information.
- Medical and surgical history.
- Hemophilia A history (blood group, and gene defect).
- Hemophilia A social history and activity level.
- Review of prior medications within the 28 days before Screening.
- Obtain blood sample for serum chemistry and hematology. Analyses should be determined at the local laboratory, and the results must be available before enrollment.
- Obtain blood sample for CD4 lymphocyte count (for HIV+ subjects; if the subject has received any blood components or per investigator discretion considering the subject's medical history).
- Vital signs.
- Physical examination.
- Record weight and height.
- Obtain blood sample for inhibitors to FVIII (analysis at central laboratory).
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins
- If FVIII activity level < 1% is not documented in subject's medical record: Obtain blood sample for plasma FVIII activity level and determine FVIII activity level at the local laboratory. The result must be available before enrollment.
- Review AEs.
- Review concomitant medications.

Subjects who complete all of these assessments and who fulfill all eligibility criteria for Arm 2 will be enrolled into the study. If the subject is not eligible for the study, the primary reason for screen failure must be entered in the eCRF.

8.10.2.1.2 Visit 1

The following procedures will be conducted and documented at Visit 1:

- If Screening and Visit 1 do not occur on the same calendar day:
  Review inclusion / exclusion criteria and reconfirm subject eligibility.
- Obtain blood sample for gene defect if sample not previously obtained or gene defect not previously documented in medical records (Note: This sample should preferably be taken at Visit 1. If this is not possible, the sample can be taken at any time during the study).
- Obtain virology retention sample.
- Hemophilia A social history and activity level.
- Record weight and height.
- Vital signs (before administration of rVIII-SingleChain).
- **Administer first rVIII-SingleChain dose.**
- If applicable: record investigator assessment of efficacy.
- Record investigator assessment of local tolerability.
- Observe subject for at least 6 hours after dosing.
- Vital signs (at 1, 2, 3, and 6 hours [± 15 minutes] after administration of rVIII-SingleChain).
- Distribute eDiary and train subject on correct use.
- Assign treatment regimen.
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- Adequate training of parents / caregivers in administration of further prophylaxis doses, identification and treatment of bleeding episodes, or provision of support to parents for training in factor administration as per standard local clinical practice. Further prophylaxis doses, or treatment doses, can be administered under supervision of the investigator as necessary. These administrations do not require further post-injection observations as above, unless clinically indicated).
- Instruct the subject and parent / caregiver on the visit schedule for the treatment period and schedule Visit 2.

8.10.2.1.3 Treatment Period

8.10.2.1.3.1 Monthly Visits from Visit 2 until 25 Exposure Days

After Visit 1, subjects on prophylaxis will have site visits **every month** (± 7 days) (Visits 2, etc) until 25 EDs with rVIII-SingleChain are achieved. The following procedures will be conducted and documented at each of these visits:

- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Obtain blood sample for serum chemistry and hematology.
- Hemophilia A social history and activity level.
- Vital signs.
- Physical examination.
- Record weight and height.
- Review subject eDiary.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes
- If rVIII-SingleChain administered at the clinic:
    - Record investigator assessment of local tolerability.
    - Record investigator assessment for treatment efficacy.
- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Perform drug accountability.
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- If treatment regimen is changed (ie, either switch to on-demand or change in prophylaxis regimen), instruct the subject and parent / caregiver on the change in visit schedule and update the eCRF (see Section 3.2.5).

8.10.2.1.3.2 Three-monthly Visits from 25 Exposure Days Until End of Study

After completion of their last monthly visit after the 25th ED, the subject will have site visits **every 3 months** (± 7 days) until End of Study. The following procedures will be conducted and documented at each of these visits:

- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Obtain blood sample for serum chemistry and hematology.
- Hemophilia A social history and activity level.
- Vital signs.
- Physical examination.
- Record weight and height.
- Review of subject eDiary.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- If rVIII-SingleChain administered at the clinic:
    - Record investigator assessment of local tolerability.
    - Record investigator assessment for treatment efficacy.
- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Perform drug accountability.
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- If treatment regimen (ie, either switch to on-demand or change in prophylaxis regimen) is changed, instruct the subject and parent / caregiver on the change in visit schedule and update the eCRF (see Section 3.2.5).

For the procedures at the End of Study Visit, see Section 8.10.6.2.

8.10.2.2 Visit Schedule for Arm 2 PUPs WITHOUT Bleeding Episode at Screening and Assigned to ON-DEMAND

8.10.2.2.1 Screening

The following procedures will be conducted and documented at Screening (within 28 days before Visit 1):

- Obtain written informed consent and assent of minors.
- Review of inclusion / exclusion criteria for Arm 2.
- Demographic information.
- Medical and surgical history.
- Hemophilia A history (blood group and gene defect).
- Hemophilia A social history and activity level.
- Review of prior medications within the 28 days before Screening.
- Obtain blood sample for serum chemistry and hematology. Analyses should be determined at the local laboratory, and the results must be available before enrollment.
- Obtain blood sample for CD4 lymphocyte count (for HIV+ subjects; if the subject has received any blood components or per investigator discretion considering the subject's medical history).
- Vital signs.
- Physical examination.
- Record weight and height.
- Obtain blood samples for
    - Inhibitors to FVIII (analysis at central laboratory).
    - Antibodies against rVIII-SingleChain and CHO proteins.
- If FVIII activity level < 1% is not documented in subject's medical record: Obtain blood sample for plasma FVIII activity level and determine FVIII activity level at the local laboratory. The result must be available before enrollment.
- Review AEs.
- Review concomitant medications.

Subjects who complete all of these assessments and who fulfill the eligibility criteria for Arm 2 will be enrolled into the study. If the subject is not eligible for the study, the primary reason for screen failure must be entered in the eCRF.

8.10.2.2.2 Visit 1

The following procedures will be conducted and documented at Visit 1:

- If Screening and Visit 1 do not occur on the same calendar day: review of inclusion / exclusion criteria for Arm 2 and reconfirm subject eligibility.
- Body weight and height.
- Obtain virology retention sample (sample must be taken before administration of first rVIII-SingleChain dose).
- Hemophilia A social history and activity level.
- Vital signs (before administration of rVIII-SingleChain).
- **Administer first rVIII-SingleChain dose.**
- Observe subject for at least 6 hours after dosing.
- Record investigator assessment of local tolerability.
- Vital signs (at 1, 2, 3, and 6 hours [± 15 minutes] after administration of rVIII-SingleChain).
- Obtain blood sample for gene defect if sample not previously obtained or gene defect not previously documented in medical records (Note: This sample should preferably be taken at Visit 1. If this is not possible, the sample can be taken at any time during the study).
- Assign treatment regimen.
- Distribute eDiary and train subject on correct use.
- Review AEs.
- Review concomitant medications.
- Adequate training of parents / caregivers in administration of rVIII-SingleChain, identification and treatment of bleeding episodes, or provision of support to parents for training in factor administration as per standard local clinical practice. Further rVIII-SingleChain doses can be administered under supervision of the investigator as necessary. These administrations do not require further post-injection observations as above, unless clinically indicated.
- Instruct the subject and parent / caregiver on the visit schedule for the treatment period and schedule the first follow-up visit.

After Visit 1, the subject can receive rVIII-SingleChain for on-demand treatment of bleeding episodes at home, if agreeable by the investigator. If home treatment is started after Visit 1, the following is to be performed:

- Distribute rVIII-SingleChain and train parents / caregivers as necessary.

8.10.2.2.3 Treatment Period

8.10.2.2.3.1 Follow-up Visits After Each rVIII-SingleChain Dose

While on an on-demand regimen, subjects will have follow-up visits **1 month (± 7 days) after each rVIII-SingleChain dose**, until End of Study.

The following procedures will be conducted and documented at each follow-up visit:

- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Obtain blood sample for serum chemistry and hematology.
- Hemophilia A social history and activity level.
- Vital signs.
- Physical examination.
- Record weight and height.
- Review of subject eDiary.
- Record investigator assessment for treatment efficacy of any bleeding episodes treated at home.
- If rVIII-SingleChain administered at the clinic: record investigator assessment of local tolerability.
- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Perform drug accountability.
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- If treatment regimen is changed, instruct the subject and parent / caregiver on the change in visit schedule and update the eCRF (see Section 3.2.5).

For the procedures at the End of Study Visit, see Section 8.10.6.2.

8.10.2.2.3.2 Monthly Telephone Calls

Subjects who do not experience a bleeding episode requiring treatment with rVIII-SingleChain within 1 month after the preceding visit will have monthly telephone calls (time window: ± 7 days) until the next bleeding episode / rVIII-SingleChain dose occurs. The following assessments will be performed at each monthly telephone call:

- Record date of telephone contact.
- Record name of staff member who contacted the subject.
- Confirm that no bleeding episodes have occurred since last visit.
- Review eDiary entries with subject.
- Review AEs.
- Review concomitant medications.
- Record data from telephone contact in eCRF.
- Arrange unscheduled visit if needed.

8.10.2.3 Visit Schedule for Arm 2 PUPs WITH Bleeding Episode at Screening and Assigned to ON-DEMAND

8.10.2.3.1 Screening

For subjects presenting with a bleeding episode at Screening, it is anticipated that the Screening and Visit 1 assessments (ie, administration of first dose of rVIII-SingleChain and relevant efficacy assessments) may occur on the same calendar day due to the emergency nature of such presentation. The following screening procedures will be completed and documented in the Screening eCRF:

- Obtain written informed consent and assent of minors.
- Review of inclusion / exclusion criteria for Arm 2.
- Demographic information.
- Medical and surgical history.
- Hemophilia A history (blood group, and gene defect).
- Hemophilia A social history and activity level.
- Review of prior medications within 28 days before Screening.
- Obtain blood sample for serum chemistry and hematology. Analyses should be determined at the local laboratory, and the results must be available before enrollment.
- Obtain blood sample for CD4 lymphocyte count (for HIV+ subjects; if the subject has received any blood components or per investigator discretion considering the subject's medical history).

- Vital signs.
- Physical examination.
- Record weight and height.
- Obtain blood samples for
    - Inhibitors to FVIII (analysis at central laboratory).
    - Antibodies against rVIII-SingleChain and CHO proteins.

  Note: rVIII-SingleChain administration does not need to be deferred until the results of inhibitor and antibodies tests are available.
- If FVIII activity level < 1% is not documented in subject's medical record: Obtain blood sample for plasma FVIII activity level and determine FVIII activity level at the local laboratory. The result must be available before enrollment.
- Review AEs.
- Review concomitant medications.

Subjects who complete all of these assessments and who fulfill the eligibility for Arm 2 will be enrolled into the study. If the subject is not eligible for the study, the primary reason for screen failure must be entered in the eCRF.

8.10.2.3.2 Visit 1

The following procedures will be conducted and documented at Visit 1:

- If Screening and Visit 1 do not occur on the same calendar day: review of inclusion / exclusion criteria for Arm 2 and reconfirm subject eligibility.
- Body weight and height.
- Obtain virology retention sample.
- Hemophilia A social history and activity level.
- Vital signs (before administration of rVIII-SingleChain).
- **Administer first rVIII-SingleChain dose.**
- Observe subject for at least 6 hours after dosing.
- Record investigator assessment of local tolerability.
- Vital signs (at 1, 2, 3, and 6 hours [± 15 minutes] after administration of rVIII-SingleChain).
- Record investigator assessment for treatment efficacy.
- Obtain blood sample for gene defect if sample not previously obtained or gene defect not previously documented in medical records (Note: This sample should preferably be taken at Screening. If this is not possible, the sample can be taken at any time during the study).

- Distribute eDiary and train subject on correct use.
- Assign treatment regimen.
- Review AEs.
- Review concomitant medications.
- Adequate training of parents / caregivers in administration of rVIII-SingleChain, identification and treatment of bleeding episodes, or provision of support to parents for training in factor administration as per standard local clinical practice. Further rVIII-SingleChain doses can be administered under supervision of the investigator as necessary. These administrations do not require further post-injection observations as above, unless clinically indicated.
- Instruct the subject and parent / caregiver on the visit schedule for the treatment period and schedule the first follow-up visit.

After Visit 1, the subject can receive rVIII-SingleChain for on-demand treatment of bleeding episodes at home, if agreeable by the investigator. If home treatment is started after Visit 1, the following is to be performed:

- Distribute rVIII-SingleChain and train parents / caregivers as necessary.

8.10.2.3.3 Treatment Period

8.10.2.3.3.1 Follow-up Visits After Each rVIII-SingleChain Dose

While on an on-demand regimen, subjects will have follow-up visits **1 month (± 7 days) after each rVIII-SingleChain dose**, until End of Study (ie, at least 75 EDs or early discontinuation).

The following procedures will be conducted and documented at each follow-up visit:

- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Obtain blood sample for serum chemistry and hematology.
- Hemophilia A social history and activity level.
- Vital signs.
- Physical examination.
- Record weight and height.
- Review of subject eDiary.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- If rVIII-SingleChain administered at the clinic: record investigator assessment of local tolerability.

- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Perform drug accountability.
- Distribute rVIII-SingleChain and train parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- If treatment regimen is changed, instruct the subject and parent / caregiver on the change in visit schedule and update the eCRF (see Section 3.2.5).

For the procedures at the End of Study Visit, see Section 8.10.6.2.

8.10.2.3.3.2 Monthly Telephone Calls

Subjects who do not experience a bleeding episode requiring treatment with rVIII-SingleChain within 1 month after their preceding visit will have monthly telephone calls (time window: ± 7 days) until the next bleeding episode / rVIII-SingleChain dose occurs. The following assessments will be performed at each monthly telephone call:

- Record date of telephone contact.
- Record name of staff member who contacted the subject.
- Confirm that no bleeding episodes have occurred since last visit.
- Review eDiary entries with subject.
- Review AEs.
- Review concomitant medications.
- Record data from telephone contact in eCRF.
- Arrange unscheduled visit if needed.

8.10.3 Visit Schedule for Arm 3 (PTPs)

8.10.3.1 Screening

The following procedures will be conducted and documented at Screening (within 28 days before Day 1):

- Obtain written informed consent and assent of minors.
- Review of inclusion / exclusion criteria for Arm 3.
- Demographic information.
- Medical and surgical history.
- Hemophilia A history (blood group, and gene defect).
- Hemophilia A social history and activity level.

- Review of prior medications within the 28 days before Screening.
- Obtain blood sample for serum chemistry and hematology.
- Obtain blood sample for CD4 lymphocyte count (for HIV+ subjects).
- Vital signs.
- Physical examination.
- Record weight and height.
- If FVIII activity level < 1% is not documented in subject's medical record: Obtain blood sample for plasma FVIII activity level and determine FVIII activity level at the local laboratory. The result must be available before enrollment.
- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Review AEs.
- Review concomitant medications.

Subjects who complete all of these assessments and who fulfill the eligibility criteria (ie, eligible subjects) for Arm 3 will be enrolled into the study. If the subject is not eligible for the study, the primary reason for screen failure must be entered in the eCRF.

8.10.3.2 Day 1

The following procedures will be conducted and documented at Day 1:

- Obtain blood sample for gene defect, if sample not previously obtained or gene defect not previously documented in medical records (Note: This sample should preferably be taken on Day 1. If this is not possible, the sample can be taken at any time during the study).
- Vital signs.
- Weight.
- Height only for subjects < 18 years of age.
- Obtain blood sample for IR.
- Obtain virology retention sample.
- Distribute eDiary and train subject on correct use.
- Assign treatment regimen (on-demand or prophylaxis).
- Distribute rVIII-SingleChain and training of parents / caregivers as necessary.
- Review AEs.
- Review concomitant medications.
- Instruct the subject and parent / caregiver on the visit schedule for the treatment period and schedule the next visit to occur 3 months ± 7 days after Day 1.

8.10.3.3 Treatment Period

Subjects in Arm 3 will visit the site **every 3 months** (± 7 days). At each visit, the following procedures will be conducted and documented:

- Hemophilia A social history and activity level.
- Serum chemistry and hematology (until 50 EDs, then yearly).
- Vital signs (until 50 EDs, then yearly).
- Physical examination (until 50 EDs, then yearly).
- Weight.
- Height only for subjects < 18 years of age.
- IR (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Inhibitors to FVIII (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Antibodies against rVIII-SingleChain (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Antibodies against CHO proteins (at the closest visits after 10 EDs, after 50 EDs, and after 100 EDs).
- Review of subject eDiary.
- Distribute rVIII-SingleChain.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- If rVIII-SingleChain administered at the clinic: record investigator assessment of local tolerability.
- Collection of used and partially used vials of rVIII-SingleChain.
- Collection of unused vials near expiration or with insufficient shelf life to cover time duration until next visit.
- Perform drug accountability.
- Review AEs.
- Review concomitant medications.

For the procedures at the End of Study Visit, see Section 8.10.6.2.

8.10.4 Follow-up Period

Not applicable.

8.10.5 Unscheduled visits

In all study arms, unscheduled visits may be arranged at any time during the study, at the discretion of the investigator or upon request of the subject or the sponsor. The assessments performed at such an unscheduled visit are at the discretion of the investigator and are to be recorded in the eCRF.

An unscheduled visits does not replace the next regular scheduled visit, unless it occurs within 7 days of the next scheduled visit.

8.10.6 End of Study

For subjects in all study arms, the End of Study Visit should take place at the end of the planned treatment period, ie:

- After at least 100 EDs in Arms 1 and 3 [PTPs].
- After 75 EDs if inhibitor negative.
- If inhibitor positive, after the 12-month post eradication follow up period or after 24 months of inhibitor treatment without CCI).
- If the subject discontinues prematurely.
- If the sponsor terminates the study.

No further study-related procedures will be performed after the End of Study Visit, except for follow-up of ongoing AEs (see Section 9.9) and inhibitor SAEs in Arm 2 PUPs (see Section 9.10).

8.10.6.1 End of Study Visit for Arm 1

In **Arm 1**, the following procedures will be performed at the End of Study Visit:

- Obtain hemophilia A social history and activity level.
- Obtain blood sample for inhibitors to FVIII.
- Obtain virology retention sample.
- Review and collect of subject eDiary.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- Collect all vials (used, partially used, and unused) of rVIII-SingleChain.
- Assess drug accountability.
- Review AEs.
- Review concomitant medications.

8.10.6.2 End of Study Visit for Arms 2 and 3

In **Arms 2 and 3**, the following procedures will be performed at the End of Study Visit:

- Obtain hemophilia A social history and activity level.
- Obtain blood sample for serum chemistry and hematology.
- Vital signs.
- Physical examination.
- Record weight and height.
- Obtain blood sample for inhibitors to FVIII.
- Obtain blood sample for antibodies against rVIII-SingleChain and CHO proteins.
- Obtain virology retention sample.
- Review and collect of subject eDiary.
- Record investigator assessment for treatment efficacy of any treated bleeding episodes.
- Collect all vials (used, partially used, and unused) of rVIII-SingleChain.
- Perform drug accountability.
- Review AEs.
- Review concomitant medications.

9 Adverse Events

9.1 Definitions

9.1.1 Adverse Event

As per the ICH guidelines, an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal, clinically significant laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

The period of observation for AEs extends from the time the subject gives informed consent until the End of Study (see Section 9.4 for further details).

AEs may include:

- Exacerbation (ie, an increase in the frequency or severity) of a pre-existing condition. Illness present before study entry should be recorded in the medical history section of the

eCRF and only be reported as an AE if there is an increase in the frequency or severity of the condition during the study.

- A clinical event occurring after consent but before rVIII-SingleChain administration.
- Intercurrent illnesses with an onset after administration of rVIII-SingleChain.

AEs do not include:

- Events identified at screening that meet exclusion criteria.
- Medical procedures (the condition that leads to the procedure is the AE).
- Situations where an untoward medical occurrence has not taken place. For example:
  - Planned hospitalizations due to pre-existing conditions, which have not worsened.
  - Hospitalizations that occur for procedures not due to an AE (eg, cosmetic surgery).
  - Hospitalizations for a diagnostic procedure where the hospital stay is less than 24 hours in duration or for normal management procedures (eg, chemotherapy).
- Overdose of rVIII-SingleChain or any concomitant therapy that does not result in any adverse signs or symptoms.
- Major or minor bleeding episodes that occur as a result of the subject's unchanged, pre-existing severe hemophilia A.

For laboratory safety parameters, any instances of absolute values being outside the reference range or changes at any visit after study start that are considered by the investigator as clinically significant must be recorded in the eCRF as AEs. In addition, at the investigator's discretion, any changes or trends over time in laboratory parameters can be recorded in the eCRF as AEs if such changes or trends are considered to be clinically relevant, even if the absolute values are within the reference range.

Laboratory findings do not need to be reported as AEs in the following cases:

- Laboratory parameters already beyond the reference range at screening, unless a further increase / decrease can be considered an exacerbation of a pre-existing condition.
- Abnormal laboratory parameters caused by mechanical or physical influences on the blood sample (eg, in vitro hemolysis) and flagged as such by the laboratory in the laboratory report.
- Abnormal parameters that are obviously biologically implausible (eg, values that are incompatible with life or outside the measuring range).
- An abnormal laboratory value that cannot be confirmed after repeat analysis, preferably in the same laboratory (ie, the previous result could be marked as not valid and should not necessarily be reported as an AE).

9.1.2 Events of Special Interest

Medical events of special interest include:

- Invasive procedures other than surgery.
- Hospital admission for less than 24 hours for events not considered as SAEs.
- Positive test result for inhibitors performed at local laboratory.

AEs of special interest include:

- Hypersensitivity reactions (including anaphylactic reactions).
- Thromboembolic events.
- Any AE related to rVIII-SingleChain associated with double or higher dose than prescribed.

9.1.3 Serious Adverse Event

An SAE is defined as any untoward medical occurrence that at any dose:

- **Results in death** – The event must be the cause of death for the SAE to meet this serious criterion.
- **Is life-threatening** – The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it had been more severe.
- **Requires in-patient hospitalization or prolongation of existing hospitalization** – CSL considers "hospitalization or prolongation of existing hospitalization" for at least 24 hours as the defining criterion for an SAE. Hospital admissions for planned surgery or for normal disease management procedures (eg, chemotherapy) are not considered as defining criteria for SAEs.
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly or birth defect.**
- **Is medically significant** – A medically significant event is defined as an event that does not necessarily meet any of the SAE criteria, but which is judged by a physician to potentially jeopardize the subject or require medical or surgical intervention to prevent one of the above outcomes listed as an SAE criterion.

AEs that do not fall into the above categories are defined as nonserious AEs.

All low-titer and high-titer inhibitors confirmed by the central laboratory are defined as SAEs (SAE criterion: "medically significant") and must be reported and followed up accordingly.

9.2 Severity of Adverse Events

The severity of each AE (ie, nonserious and serious AEs) is to be assessed by the investigator as follows:

Table 10: Severity of Adverse Events

Severity	Definition
Mild	A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Moderate	A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
Severe	A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Abbreviations: AE = adverse event; CDISC = Clinical Data Interchange Standards Consortium; SDTM = Severity Intensity Scale for Adverse Event Terminology.

9.3 Causality of Adverse Events

The causal relationship of an AE to rVIII-SingleChain must always be assessed by the investigator. All AEs will be classified as either **related** or **not related** to rVIII-SingleChain. If a causality assessment is not provided for an AE (including an SAE), that AE will be considered related to rVIII-SingleChain.

The degree of certainty with which an AE is attributed to rVIII-SingleChain or an alternative cause (eg, natural history of the underlying disease, concomitant therapy) will be determined by how well the event can be understood in terms of:

- Known pharmacology of rVIII-SingleChain.
- Clinically and / or pathophysiologically plausible context.
- Reaction of a similar nature previously observed with similar products, or reported in the literature for similar products as being product related (eg, headache, facial flushing, pallor).
- Plausibility supported by the temporal relationship (eg, the event being related by time to administration or termination of treatment with rVIII-SingleChain, drug withdrawal or reproduced on rechallenge).

9.4 Observation Period for Adverse Events

The observation period for AE (and SAE) reporting in an individual subject will start at the time of giving written informed consent for participation in the current study and finish with the End of Study Visit.

If the investigator becomes aware of an SAE that has started after the observation period has finished, and the event could in some way be associated with rVIII-SingleChain (irrespective of whether or not it is considered by the investigator to be causally related to rVIII-SingleChain), then this must also be reported to CSL and included in the clinical database (see Section 9.6).

9.5 Adverse Event Reporting

9.5.1 Adverse Events

At each clinical evaluation, the investigator (or delegate) will determine whether any AEs have occurred. AEs will be recorded in the AE page of the eCRF. If known, the medical diagnosis of an AE should be recorded in preference to the listing of individual signs and symptoms. The investigator must follow up on the course of an AE until resolution or stabilization. If an AE is ongoing after the End of Study Visit, the AE will continue to be followed up until resolution, stabilization, or 28 days after the final administration of rVIII-SingleChain, whichever is sooner. Inhibitor SAEs in Arm 2 (PUPs) will be followed up for 28 days after the End of Study Visit (see Section 9.10).

If, during the study period, a subject presents with a preexisting condition that was not noted at the time of study entry, the condition should be retrospectively recorded in the Medical History section of the eCRF.

9.5.2 Events of Special Interest Reporting

Any AE of special interest or medical event of special interest will be recorded in the eCRF and will be automatically reported to the sponsor via email alert.

9.6 Serious Adverse Event Reporting

This study will comply with all applicable regulatory requirements and adhere to the full requirements of ICH Topic E2A (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

For SAEs occurring during the study, the investigator or delegate will enter all relevant information in the AE page of the eCRF. An electronic document containing the AE page and other applicable pages of the eCRF must be sent (via facsimile or email) to the sponsor together with a **Notification of Serious Adverse Event at Investigator Site** cover page, which has been signed and dated by the investigator. If an electronic document is not able to be generated (eg, internet access problem), a handwritten paper SAE report must be completed, which must be signed and dated by the investigator.

All SAEs that occur during the course of the study, whether or not causally related to rVIII-SingleChain, must be reported immediately (within 24 hours of the investigator becoming aware of the event) to CSL. For Japan sites only, the investigator must also inform the head of the medical institution of the SAE and related information in accordance with the Japanese regulatory requirements and ICH GCP.

AEs occurring in the period between the times the subject gave written informed consent and the first exposure to rVIII-SingleChain that meet one or more of the seriousness criteria for AEs must be reported to CSL in the same manner as other SAEs and will be included in the clinical study database.

Any SAE that occurs after the End of Study Visit that is considered to be causally related to rVIII-SingleChain must be **reported immediately (ie, within 24 hours of the investigator becoming aware of the event) to CSL**. For Japan sites only, the investigator must also inform the head of the medical institution of the SAE and related information in accordance with the Japanese regulatory requirements and ICH GCP.

Contact details and guidance for reporting SAEs will be provided to study site before the study site is initiated.

9.6.1 Requirements for Immediate Reporting of Serious Adverse Events

The minimum reporting requirements for immediate reporting of SAEs include:

- Identifiable subject.
- Suspected medicinal product and / or procedure.
- Event term.
- Identifiable reporting source.

In addition, the investigator must:

- Report all SAEs to the relevant institutional review board (IRB) / independent ethics committee (IEC) within the timeframe specified by the IRB / IEC.
- Submit follow-up reports to CSL Clinical Safety and Pharmacovigilance until the SAE has resolved, or, in the case of permanent impairment, until stabilized.
- Ensure that the causality assessment for all SAEs is entered in the eCRF.

If the minimum requirements for reporting are fulfilled, the investigator should not wait to receive additional information to fully document the event before notifying CSL.
When submitting SAE reports and any other related reports (eg, discharge summaries) to CSL, subjects should be identified only by their subject number and study number. The investigator should not include the subject's name, date of birth, or address.

In cases of death, the investigator should supply CSL and the IEC / IRB (as applicable) with any additional information as it becomes available (eg, autopsy reports and detailed medical reports).

The procedure to be followed if an ongoing AE becomes an SAE after the end of the observation period for AEs is described in Section 9.9.

9.7 Other Significant Event Reporting

9.7.1 Overdose

Details (ie, volume, location of injections, injection rate) of an overdose of rVIII-SingleChain or any concomitant therapy must be recorded in the eCRF. Any overdose that is considered by the investigator to be medically significant must be reported as an SAE (see Section 9.6).

Any AE related to rVIII-SingleChain associated with ≥ 2 times the prescribed dose is considered an overdose.

9.7.2 Pregnancy and Lactation

A female partner of a male subject who becomes pregnant while participating in the study, or up to and including 28 days after the last dose of rVIII-SingleChain, must notify the investigator immediately.

CSL must be notified within 5 days of the investigator becoming aware of the pregnancy.

Whenever possible, a pregnancy in a female partner of a male subject exposed to rVIII-SingleChain should be followed to term so as to assess any potential occurrence of congenital anomalies or birth defects. Any follow-up information, including premature termination and the status of the mother and child after delivery, should be reported by the investigator to CSL using a Pregnancy Reporting / Outcome Form.

9.8 IRB / IEC Reporting Requirements

The time frame within which an IRB / IEC must be notified of deaths and investigational product-related unexpected SAEs is stipulated by each IRB / IEC. It is the investigator's responsibility to comply with the requirements for IRB / IEC notification. CSL will provide investigators with all details of all SAEs reported to regulatory authorities.

9.9 Follow-up of Adverse Events

Every effort should be made to follow-up subjects who continue to experience an AE or an SAE on completion of the study until either the AE resolves or stabilizes, or until 28 days after the final administration of rVIII-SingleChain, whichever is sooner. All follow-up information (and attempted follow-up contacts) should be documented in the subject's medical records. Details of the subject's progress should also be submitted to CSL Global Clinical Safety and Pharmacovigilance.

For the specific follow-up procedures for inhibitor SAEs in Arm 2 (PUPs) after the End of Study Visit, see Section 9.10.

9.10 Follow-up of Inhibitor SAEs in Arm 2 (PUPs)

PUPs in Arm 2 who develop an inhibitor SAE (ie, low-titer or high-titer inhibitors confirmed by the central laboratory) will be followed up for **28 days after their End of Study Visit**. All follow-up information (and attempted follow-up contacts) should be documented in the subject's medical records and submitted to CSL Global Clinical Safety and Pharmacovigilance. At a minimum, the following specific information should be documented and submitted:

- Information on last inhibitor titer.
- Details and outcome of major / life-threatening bleeding episodes.

- Hemophilia treatment since End of Study Visit:
  - Treatment modality (on-demand, prophylaxis).
  - Currently receiving CCI [redacted]
  - CCI [redacted]
  - Treatment with bypassing agents (yes, no).
- AEs since End of Study Visit.
- Development of inhibitor after previous eradication. In the event of inhibitor development after previous eradication, this will be considered a new SAE and should be reported as such.

10 Assessments

10.1 Subject Characteristics

Subject characteristics to be evaluated will include:

- Demographic data (eg, sex, race, height, body weight).
- Medical and surgical history.
- History of hemophilia A.

10.2 Efficacy Assessments

The efficacy of rVIII-SingleChain treatment in the prophylaxis and treatment of bleeding episodes will be assessed based on the following:

- Total consumption of rVIII-SingleChain (number of EDs and mean IU/kg per year per subject and per bleeding episode) and consumption per routine prophylaxis, on-demand treatment, and surgery.
- Number of traumatic and non-traumatic bleeding episodes.
- Investigator's clinical assessment of hemostatic efficacy for treatment of bleeding episodes (4-point scale).
- Number of rVIII-SingleChain injections required to achieve hemostasis.
- Investigator's clinical assessment of hemostatic efficacy for treatment during surgery (4-point scale).

10.3 Safety Assessments

Safety will be assessed based on the following variables:

- AEs and SAEs.
- Serum chemistry and hematology.
- Physical examination.
- Vital signs.
- Inhibitors to FVIII.
- Antibodies against rVIII-SingleChain.
- Antibodies against CHO proteins.
- Subject / caregiver assessment of local tolerability.
- **Arms 2 and 3 only**: investigator assessment of local tolerability for rVIII-SingleChain injections administered at the clinic.

Clinical laboratory tests will be performed at timepoints as detailed in the Schedules of Assessments. More frequent evaluations may be performed, if clinically indicated, at the discretion of the investigator.

10.4 Pharmacokinetic and Pharmacodynamics

- IR (see Section 8.6).

10.5 Other Assessments

- FVIII activity level at Screening (Arms 2 and 3), if not available in subject's medical records. FVIII activity level must be determined at the local laboratory, and the result must be available before enrollment.

11 Statistics

11.1 Sample Size Estimation

The sample size was based on the EMA guidelines for the clinical investigation of recombinant and human plasma-derived factor VIII products [EMA, 2011]. Therefore, no sample size calculations were performed. The target enrollment is at least 200 PTPs (Arms 1 and 3) completing at least 100 EDs during enrollment in all CSL-sponsored rVIII-SingleChain studies and 24 PUPs (Arm 2) completing 50 EDs of rVIII-SingleChain in this study. Thus, the study will enroll at total of least 224 subjects with severe hemophilia A.

11.2 Analysis Populations

All analyses in all analysis populations will be performed separately for PTPs and PUPs.

11.2.1 Enrolled Population

The Enrolled population will comprise all subjects who provided informed consent and were not screen failures.

11.2.2 Safety Population

The safety population will comprise all subjects in the Enrolled population who received at least 1 dose of rVIII-SingleChain during the study for any reason (eg, surgery, routine prophylaxis, on-demand treatment).

11.2.3 Efficacy Population

The efficacy population will consist of all subjects in the safety population who receive at least 1 dose of rVIII-SingleChain as part of either routine prophylaxis treatment or on-demand treatment during the study. Additionally, the efficacy population will only include subjects who do not have an inhibitor.

The Efficacy population might be the same as the Safety population.

11.2.4 Surgery Population

The Surgery population will include all subjects in the Safety population who received at least 1 dose of rVIII-SingleChain for surgical prophylaxis.

CCI

11.2.6 Pharmacokinetic Population

Not applicable.

11.2.7 Pharmacodynamic Population

Not applicable.

11.3 Statistical Analyses and Methods

All safety and efficacy data will be summarized or listed as appropriate. Continuous data will be summarized using descriptive statistics and categorical data will be summarized using frequency counts and percentages. There are no plans to produce p values. Confidence intervals (CIs) will be provided for key parameters where noted here or in the final statistical analysis plan. Except where otherwise noted, the CIs will be two-sided at the 95% level.

All analyses will be performed separately for PTPs and PUPs, including the analyses in the surgical substudy.

A complete description of the statistical analyses and methods will be available in the statistical analysis plan, which will be finalized before the database is locked.

11.3.1 Subject Disposition and Characteristics

11.3.1.1 Subject Disposition

The number of subjects who enrolled into this study, completed the study, withdrew from the study or discontinued the IMP will be presented in summary tables. The reason for discontinuing the IMP or withdrawing a subject from the study will be summarized.

The same methods will be applied to the substudies.

11.3.1.2 Subject Characteristics

Demographics and baseline subject characteristics will be presented in summary tables and data listings. Age will be described as both a continuous and a discrete variable. Supportive data will be listed by subject. A listing of gene abnormalities by subject will be prepared.

11.3.2 Efficacy Analyses

The efficacy data from the main study will be summarized and analyzed for the efficacy population unless otherwise specified. The surgery substudy efficacy data will be summarized for the surgery population.

11.3.2.1 Primary Efficacy Analysis

For **Arms 1 and 3 (PTPs)**, there is no primary efficacy endpoint.

For **Arm 2 (PUPs)**, the analyses of the primary efficacy endpoints are outlined below:

- The investigator's clinical assessment of hemostatic efficacy for the treatment of **major** bleeding episodes, based on the 4-point ordinal scale (excellent, good, moderate, poor / no response), will be tabulated. The percentage of major bleeding episodes treated successfully (defined as ratings of excellent or good) will be summarized, and reported together with a two-sided 95% CI. To estimate the proportion, the denominator will include all treated bleeding episodes categorized as major. To account for within-subject correlation, generalized linear modeling using SAS' GENMOD procedure will be utilized. The model will contain only the intercept term. The binomial distribution with logit link function will be specified.
- The AsBR will be derived for each subject as follows: ((number of spontaneous bleeding episodes) / (observed treatment period of interest))*365.25. The AsBR will be summarized by regimen (on-demand or prophylaxis) using descriptive statistics. In addition, the number of spontaneous bleeding episodes per year and associated 95% CI will be estimated based on a Poisson model. Generalized linear modeling using SAS' GENMOD procedure will be utilized. The Poisson distribution with log link function, and log_time offset will be specified. The Poisson-based number of spontaneous bleeding episodes per year will be presented by regimen (on-demand or prophylaxis).

11.3.2.2 Secondary Efficacy Analyses

- In **all study arms**, the analyses of the secondary efficacy endpoints are as follows: The primary efficacy analyses outlined in Section 11.3.2.1 for the AsBR will be repeated for the ABR (traumatic, non-traumatic, and unknown).
- The number of bleeding episodes and the number of treated bleeding episodes will be presented. The number and percentage of bleeding episodes requiring 1, 2, 3, or > 3 injections of rVIII-SingleChain to achieve hemostasis will be tabulated. The total dose per bleeding episode (IU/kg) will be summarized using descriptive statistics.
- The consumption of rVIII-SingleChain, will be derived and expressed in terms of IU/kg per month, IU/kg per year, total IU per injection, per month and per year. Consumption will be summarized using descriptive statistics for on-demand, prophylaxis, and surgery.
- The investigator's clinical assessment of hemostatic efficacy for surgery based on the 4-point ordinal scale will be tabulated.

In **Arms 1 and 3 (PTPs),** the following analysis will be performed in addition to those above:

- The investigator's clinical assessment of hemostatic efficacy for the treatment of bleeding episodes, based on the 4-point ordinal scale (excellent, good, moderate, poor / no response), will be tabulated by severity (major and non-major). The percentage of bleeding episodes treated successfully, defined as excellent or good) will be summarized and reported together with a two-sided 95% CI. In order to account for within-subject correlation, generalized linear modeling using SAS' GENMOD procedure will be utilized. The model will contain only the intercept term. The binomial distribution with logit link function will be specified.

In **Arm 2 (PUPs)**, the following analysis will be performed in addition to those above:
The investigator's clinical assessment of hemostatic efficacy for the treatment of **non-major** bleeding episodes will be analyzed as described in Section 11.3.2.1 for major bleeding episodes

Details on the analyses for the secondary efficacy endpoints will be provided in the Statistical Analysis Plan (SAP).

11.3.2.3 Other Analyses

The characteristics of bleeding episodes including the type of event (traumatic or non-traumatic or unknown causality) and the location of bleeding will be summarized using frequency counts and percentages. The actual dose (IU/kg) of rVIII-SingleChain, the time between start of bleeding to the first rVIII-SingleChain injection.

Surgery data will be presented including the type of surgery (emergency or planned), the relationship to hemophilia (related or not related), rVIII-SingleChain consumption (including actual dose regimen, actual IU per dose, and number of injections per day and pre-, intra-, and post-surgery), and complications.

11.3.3 Safety Analyses

Safety summaries will be performed on the safety population unless otherwise specified. For the surgery and CCI substudies, safety summaries will be performed on the respective substudy populations.

11.3.3.1 Primary Safety Endpoint

In **Arms 1 and 3 (PTPs),** the primary safety endpoint is the incidence of inhibitor formation to FVIII in at least 200 PTPs with at least 100 EDs of rVIII-SingleChain.

In **Arm 2 (PUPs)**, the primary safety endpoint is the incidence of high-titer inhibitor formation to FVIII in PUPs with at least 50 EDs of rVIII-SingleChain.

11.3.3.2 Primary Safety Analysis

The incidence of inhibitor formation to FVIII, based on the number of EDs and subjects specified in Section 11.3.3.1 will be reported together with a (2-sided) 95% CI. However, if no inhibitor formation is observed, then the upper one-sided 97.5% confidence limit will be reported. For calculation of the incidence for each study arm, the numerator will include all subjects who develop inhibitors in each arm and the denominator will include:

a) all PTPs with at least 100 EDs plus PTPs with less than 100 EDs but with inhibitors.
b) all PUPs with at least 50 EDs plus PUPs with less than 50 EDs but with high-titer inhibitors.

11.3.3.3 Secondary Safety Endpoints

See Section 2.2.2.2.

11.3.3.4 Secondary Safety Analysis

11.3.3.4.1 Incidence of Inhibitor Formation to FVIII at Intermediate Timepoints of Exposure to rVIII-SingleChain

Arms 1 and 3 (PTPs)

To calculate the inhibitor incidence after 10 EDs (after 50 EDs, and after 100 EDs), the numerator will include all subjects with inhibitors observed at the closest visit up to 10 EDs (≤ 10 EDs) (up to 50 EDs, and up to 100 EDs). The denominator will include all subjects with at least 10 EDs (≥ 10 EDs) (at least 50 EDs, and at least 100 EDs) plus subjects with less than 10 EDs (< 10 EDs) (less than 50 EDs, and less than 100 EDs) but with inhibitors.

Arm 2 (PUPs)

Inhibitor incidence in PUPs will be analyzed analogously to PTPs. The same principles will be applied, taking into account: number of subjects at risk for the relevant number of EDs (after 10 EDs and after 50 EDs) and inhibitor type (low, low + high).

11.3.3.4.2 Antibodies Against rVIII-SingleChain and Antibodies to CHO Proteins

In all study arms, the occurrence of antibodies against rVIII-SingleChain will be summarized by visit using frequency counts and percentages. The denominator will include the number of subjects in the safety population.

The occurrence of antibodies to CHO proteins will be summarized by visit and using frequency counts and percentages. The denominator will include the number of subjects tested at each visit.

11.3.3.4.3 Clinically Significant Abnormal Vital Signs (Arm 2 Only)

The number of PUPs with clinically significant abnormal values in vital signs assessments in the main study will be summarized by assessment timepoint and by variable (heart rate, blood pressure, body temperature). The overall percentage of PUPs with clinically significant abnormal values in any of the vital signs variables a) after the first rVIII-SingleChain injection and b) at any assessment timepoint during the study will be calculated. PUPs who experience more than 1 abnormal value will only be counted once for the analysis of these endpoints.

11.3.3.5 CCI

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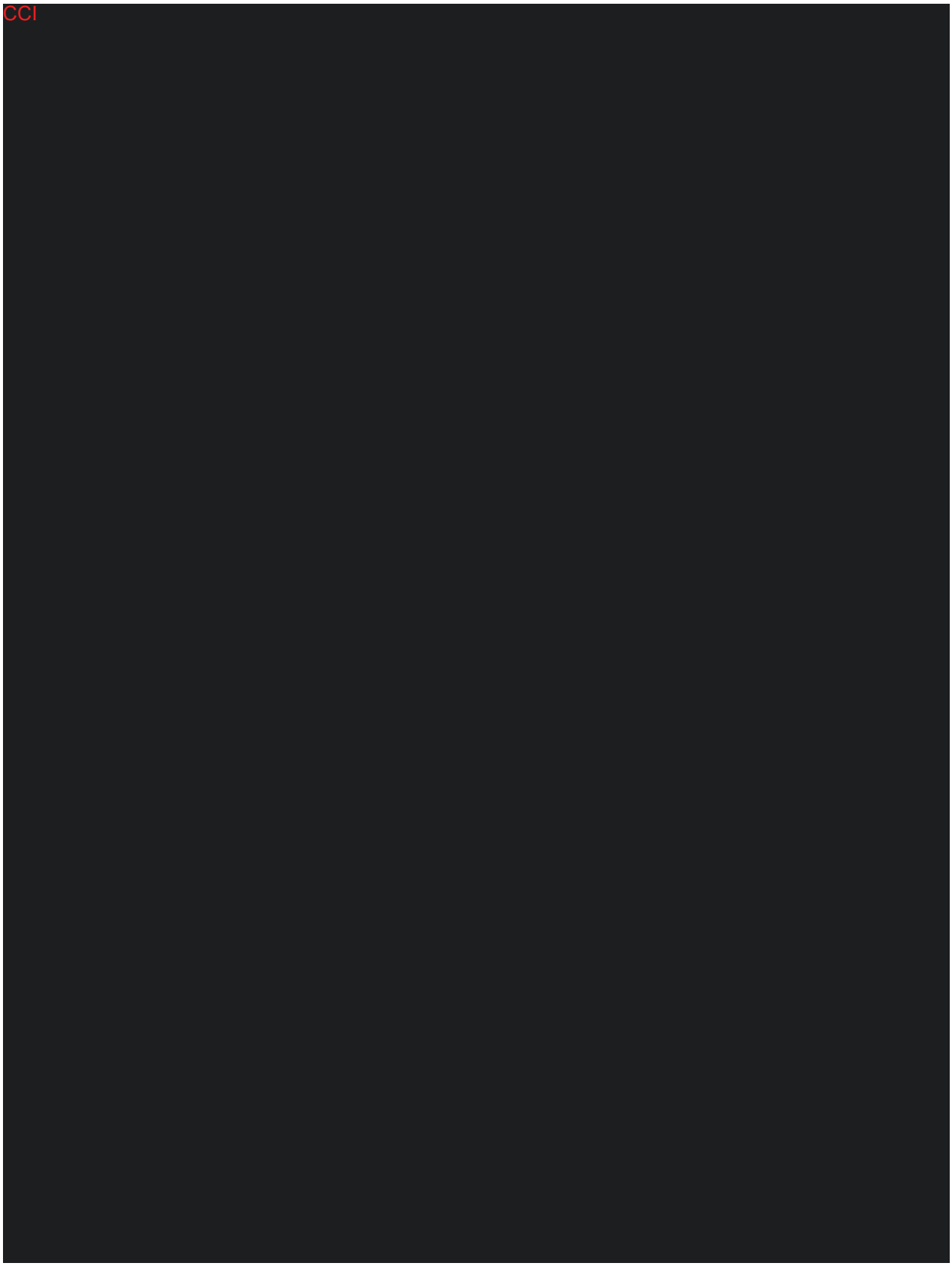

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11.3.4 Pharmacokinetics and Pharmacodynamic Analyses

Incremental recovery is defined as FVIII activity obtained 30 minutes after injection and divided by dose of rVIII-SingleChain (IU/mL / IU/kg). Incremental recovery values will be predose-corrected for pre-injection plasma FVIII activity. The exact times of blood draws after injection will be recorded and used in the analysis. Incremental recovery will be listed and summarized by assessment timepoint, using descriptive statistics.

11.3.5 Other Analyses

11.3.5.1 Adverse events

AEs will be recorded from the time of informed consent until End of Study Visit (ie, at study completion or at early discontinuation). Treatment-emergent AEs (TEAEs) for this study are defined as AEs with an onset during or after the first rVIII-SingleChain injection in this study.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 21.0. The number and percentage of subjects who experience AEs will be summarized overall by system organ class (SOC) and preferred term (PT) and will also be summarized based on maximum severity and relationship to rVIII-SingleChain.

Descriptive analysis of AEs will include:

- Incidence of TEAEs, AESIs, and SAEs grouped by SOC and PT.
- Incidence of TEAEs by seriousness; incidence of non-serious AEs by pre-defined thresholds.
- Incidence of TEAEs, grouped by SOC and PT, with reference to the relationship to rVIII-SingleChain and maximum severity.
- Incidence of TEAEs leading to withdrawal grouped by SOC and PT.
- Incidence of related AEs to rVIII-SingleChain over the course of the study.

Individual listings of all AEs will also be provided.

All data will be analyzed and presented separately for PTPs and PUPs. SAEs, deaths, and discontinuations due to AEs will be summarized and supported by listings.

All data will also be analyzed and presented separately for the surgery substudy and the CCI substudy, ie, counting only AEs with an onset between enrolment into the substudy and the End of Substudy Visit. CCI [redacted]

11.3.5.2 Local tolerability

Results of the local tolerability assessments (Arm 1: subject / caregiver assessment only; Arms 2 and 3: subject / caregiver and investigator assessments) will be summarized using frequency counts and percentages. The number of subjects with at least 1 assessment, as well as the total number of assessments will be presented. The percentage of subjects with assessments in each category will be presented, as well as the percentage of all assessments in each category will be presented.

11.3.5.3 Subgroup Analyses

The incidence of inhibitors will be summarized by race (White, Black, Asian, and Other). Any additional subgroups will be defined in the SAP. These summaries will be provided separately for low-titer inhibitors, high-titer inhibitors, and overall for each study arm.

For the surgery substudy, no predefined subgroup analyses will be performed.

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11.3.6 Interim Analysis

No formal interim analyses are planned. The final analysis of PTPs will be conducted when all PTPs have completed the study to support the production of a study report on PTPs before the reporting for PUPs. In addition, safety, efficacy and / or PK data may be reported to regulatory authorities while the study is ongoing to supplement information from completed lead-in studies or the ongoing study. Those analyses do not have an impact on the overall study design or further study conduct, and therefore are not considered to be interim analysis in the sense of ICH E9.

12 Quality Assurance

The study may be subject to an audit by CSL, an authorized representative(s) of CSL and / or inspections by an authorized regulatory authority (eg, US Food and Drug Administration [FDA]). Regulatory authorities may request access to all study documentation, including source documents for inspection and copying, in keeping with local regulations. CSL will immediately notify the investigator of an upcoming audit / inspection.

In the event of an audit, all pertinent study-related documentation must be made available to the auditor(s). If an audit or inspection occurs, the investigator at each study site will permit the auditor / inspector direct access to all relevant documents and allocate their time as well as the time of relevant staff to discuss the findings and any relevant issues.

13 Regulatory and Ethics Considerations

13.1 Regulatory Considerations

CSL or its agents will submit the appropriate documents to the local regulatory agencies and will await approval before study start.

This study will be conducted under a US FDA Investigational New Drug application and also in other regions and documented in accordance with the applicable regulatory guidelines and requirements.

The procedures set out in this study protocol are designed to ensure that CSL and the investigator abide by the principles of the current ICH GCP guideline on the conduct, evaluation and documentation of this study, as described in ICH Topic E6 (Guideline for GCP). The study will also be carried out according to all applicable international and national regulatory requirements.

13.2 Institutional Review Board / Independent Ethics Committee

The investigator must submit the protocol and informed consent forms (ICFs) for review by an authorized and properly constituted (according to local guidelines) IRB / IEC. Written approval must be received from the IRB / IEC before commencement of the study.

13.3 Subject Information and Informed Consent

The principles of informed consent and guardian assent in the Declaration of Helsinki must be implemented in this clinical study before protocol-specified procedures are carried out.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB / IEC. Subjects, their relatives (or if necessary, legally acceptable representatives) must be given ample opportunity to inquire about details of the study.

Should there be any amendments to the protocol that would directly affect the subject's participation in the study (eg, a change in any procedure); the ICF must be amended to incorporate this modification. Subject must be informed of the change and they must sign the amended ICF indicating that they re-consent to participate in the study.

13.4 Subject Identification and Confidentiality

All subject names and contact details will be kept confidential. Subjects will be identified throughout documentation and evaluation by the number allotted to them during the study. Each subject will be told that all study findings will be handled in the strictest confidence.

The investigator at the study site will be responsible for retaining sufficient information about each subject (eg, name, address, phone number and identity in the study) so that regulatory agencies or CSL may access this information should the need arise. These records should be retained in a confidential manner as long as legally mandated according to local requirements.

Subject medical records pertaining to the study may be inspected / audited at any time by CSL employees or their duly authorized representatives, a regulatory authority or the IRB / IEC. All records accessed will be strictly confidential. Consent to participate in the study includes consent to these inspections / audits.

13.5 Indemnity and Compensation

It is CSL policy that persons who participate in CSL's clinical studies should be no worse off for their having been involved in the study. These persons include the subjects / volunteers, the investigator, the hospital and the IRB / IEC.

CSL has taken out insurance to cover its obligations under both the Indemnity and the Compensation guidelines for injury to subjects involved in the study.

Other details regarding compensation and the obligations of the investigator / CSL are provided in the Clinical Trial Agreement for the study (see Section 14.1).

14 Administrative Considerations

14.1 Clinical Trial Agreement

This study will be conducted under a Clinical Trial Agreement between CSL ("Sponsor") and the institution(s) representing the investigational study site(s) ("Authority"). Financial support to the investigational site(s) will be detailed in the Clinical Trial Agreement. The Clinical Trial Agreement must be signed before the commencement of the study and will clearly delineate the responsibilities and obligations of investigator and CSL, and will form the contractual basis under which the clinical study will be conducted.

14.2 Clinical Study Registration and Results Disclosure

CSL will provide the relevant study protocol information in public database(s) before or at commencement of the study. CSL may also provide study information for inclusion in national registries according to local regulatory requirements.

Results of this study will be disclosed according to the relevant regulatory requirements. All publications in peer-reviewed medical journals resulting from this study will be listed in the original study protocol registration record.

14.3 Implementation of the Protocol / Protocol Amendment(s)

With the exception of medical emergencies, no changes or deviations in the conduct of the signed protocol will be permitted without documented approval of the CSL Medical Monitor and the IRB / IEC. In the event of a medical emergency, the investigator at the study site will institute any medical procedures deemed appropriate. However, all such procedures must be promptly reported to the CSL Medical Monitor and the IRB / IEC.

Modifications to the protocol that may affect subject safety or the way the study is to be conducted will be documented in a protocol amendment, which must be approved by the IRB / IEC.

Administrative changes to the protocol, defined as minor corrections and / or clarifications that have no effect on the way the study is to be conducted, will not require IRB / IEC approval, but will be submitted to the IRB / IEC for their information.

14.4 Protocol Deviations

All instances where the requirements of the study protocol were not complied with will be tracked. Corresponding subjects may be withdrawn from the study at the discretion of the investigator and / or CSL. Study protocol deviations arise when subjects who have been entered in the study deviate from the IEC / IRB-approved study protocol.

If a major protocol deviation (ie, a deviation that could have a significant effect on the subject's safety, rights, or welfare and / or on the integrity of the study data) occurs, the investigator must notify CSL and the appropriate IRB / IEC as soon as possible or as per local requirements.

14.5 Documentation and Record Keeping

14.5.1 Data Collection

The investigator (or delegate) will maintain individual records for each subject. These records should include dates when a subject visited the study site, records of vital signs, medical history, or physical examinations, administration of rVIII-SingleChain or concomitant therapy, any AEs experienced, and other notes as appropriate. These records constitute source data.

An eCRF will be provided by CSL (or delegate) for each subject enrolled into the study. The investigator is responsible for ensuring accurate and proper completion of the eCRF in a timely manner so that it always reflects the latest observations on the subjects enrolled in the study. All entries on the eCRF must be backed up by source data unless there is prior agreement that the eCRF is the source data.

All source data will be kept according to all applicable regulatory requirements. Source data must be completed legibly for each subject enrolled into the study and signed by the investigator (or delegate).

The subject diary will be completed by the subject at home. At each visit, the subject will present the completed diary to the investigator for review of entries and transfer of relevant information to the eCRF.

14.5.2 Data Quality Assurance

Data generated throughout the study will be monitored and the eCRFs checked against the subject records for completeness and accuracy. CSL's study monitor will perform this function.

Following completion of eCRF pages and entry of the data into a database, the data will be checked electronically for consistency and plausibility. Queries will be generated for questionable data and clarification sought from the investigator. These data queries must be resolved in a timely manner by the investigator (or delegate).

14.5.3 Record Retention

An investigator study file prepared by CSL (or delegate), containing all applicable documents for use at the study site, will be made available to the investigator before the start of the study. All study documentation and materials maintained in the investigator study file must be kept in conformance with applicable national laws and regulations.

All study documentation and materials maintained in the investigator study file at the study site must be available for inspection by the CSL's study monitor (or delegate) to determine that all required documentation is present and correct.

The study may be audited or inspected by qualified delegates from CSL or a competent regulatory authority.

Following completion of the study, the investigator is responsible for archiving the investigator's study file, the subject's records and the source data according to applicable regulatory requirements.

14.6 Study and Site Closure

CSL reserves the right to prematurely discontinue or suspend the study (or 1 or more of the study arms, or the substudies) either at a particular site or at all study sites at any time and for any reason. If such action is taken, the CSL Study Monitor (or delegate) will discuss this with the investigator (for Japan sites only: the head of the medical institute) at each study site at that time and notify the investigators in writing. If the study is suspended or terminated for safety reasons, all investigators (for Japan sites only: the heads of the medical institutes) and the relevant regulatory agencies will be immediately notified of the action, as well as the

reason for it. The investigator (for Japan sites only: the head of the medical institute) at each study site will advise the IRB / IEC overseeing the study at their site.

14.7 Clinical Study Report

A final clinical study report will be written after the completion of the study. CSL or its agent will write the report in consultation with the investigator or, if applicable, a nominated coordinating investigator (or delegate). It is required by CSL that the coordinating investigator will sign the final clinical study report.

Progress reports may be provided to the relevant regulatory bodies in accordance with their requirements.

14.8 Use of Data and Publications

The rights and obligations of investigators and CSL concerning any formal presentation or publication of data collected as a direct or indirect result of this study will be addressed specifically in the Clinical Trial Agreement for the study.

15 References

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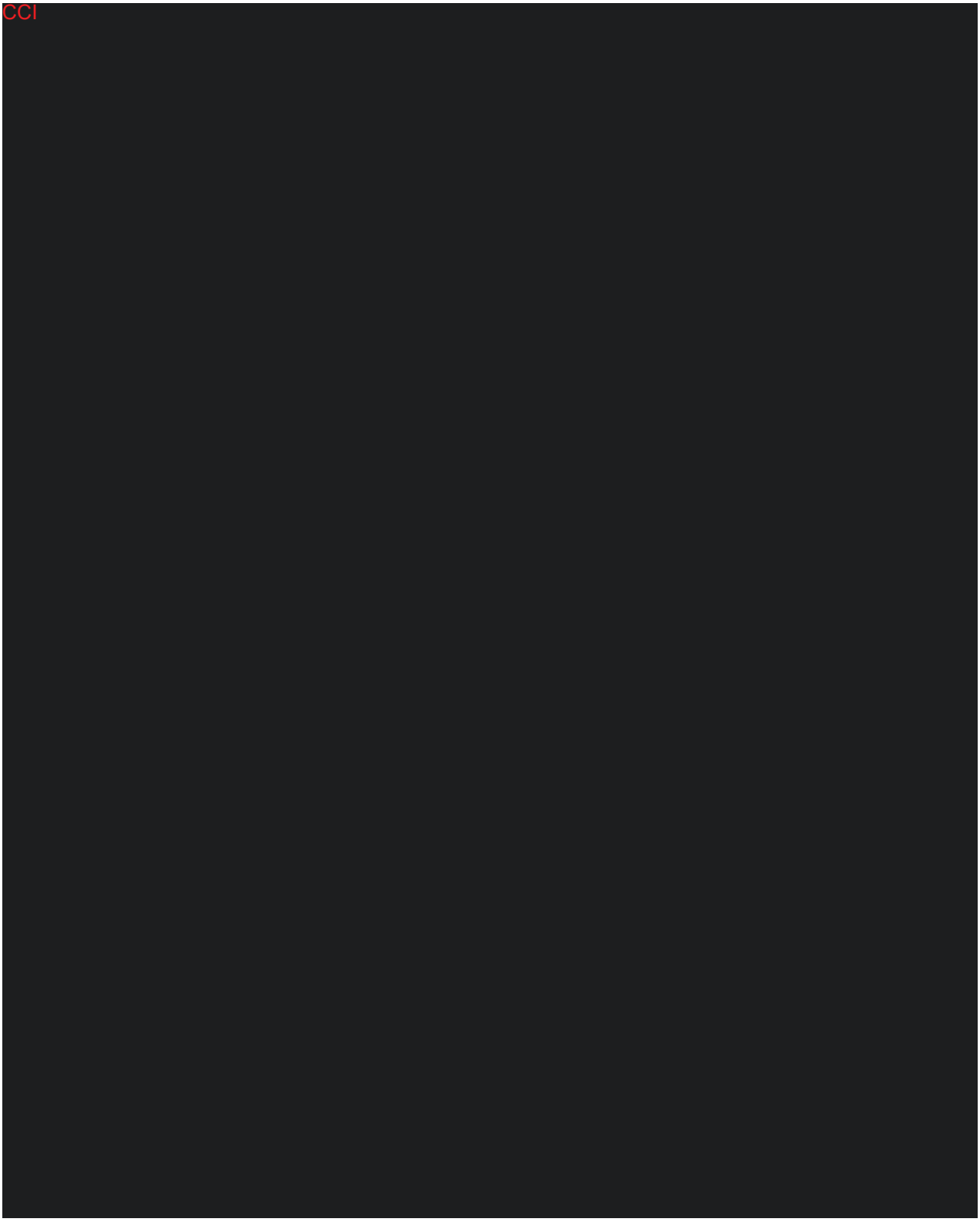
Appendix 1: CCI



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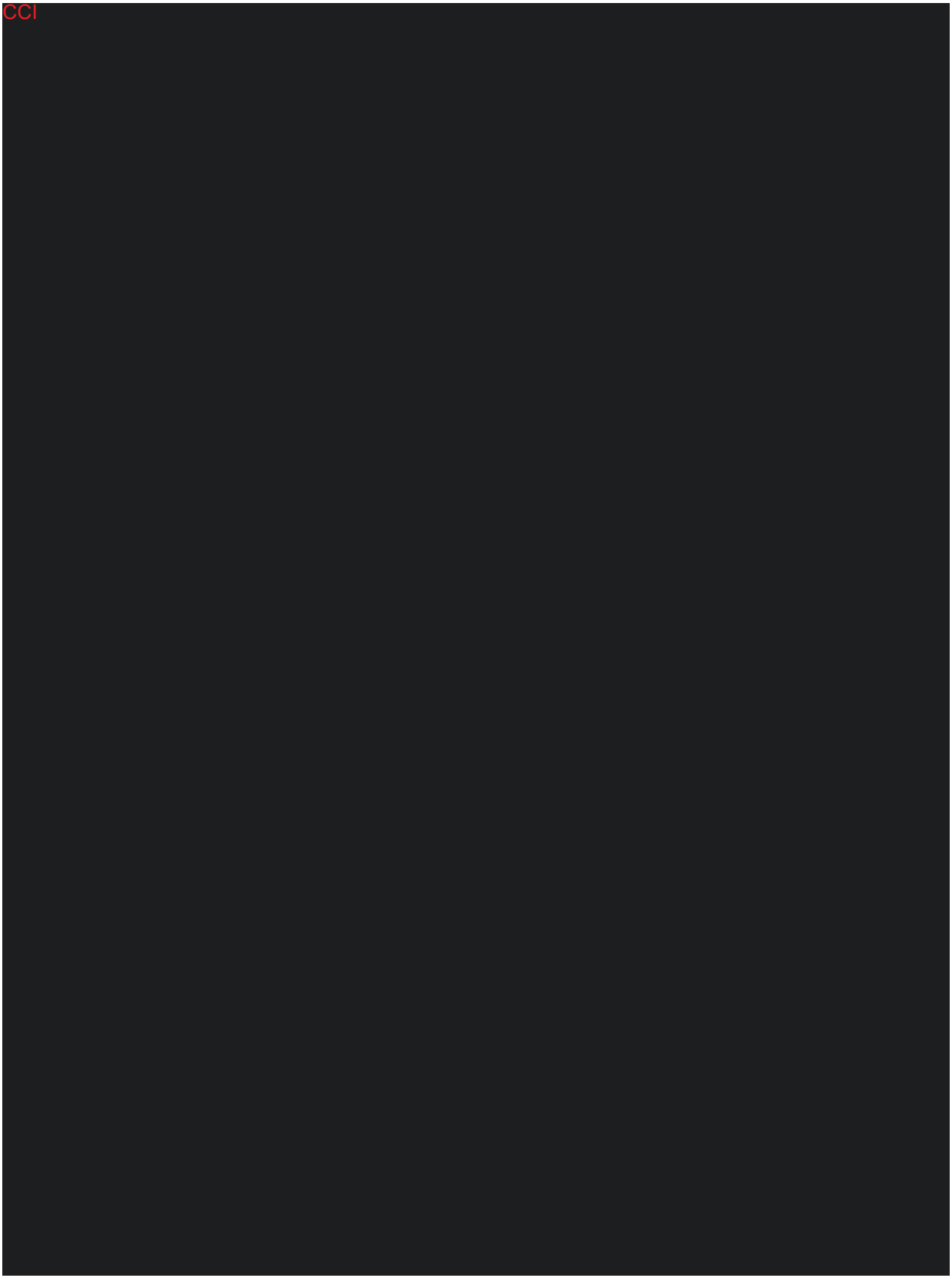

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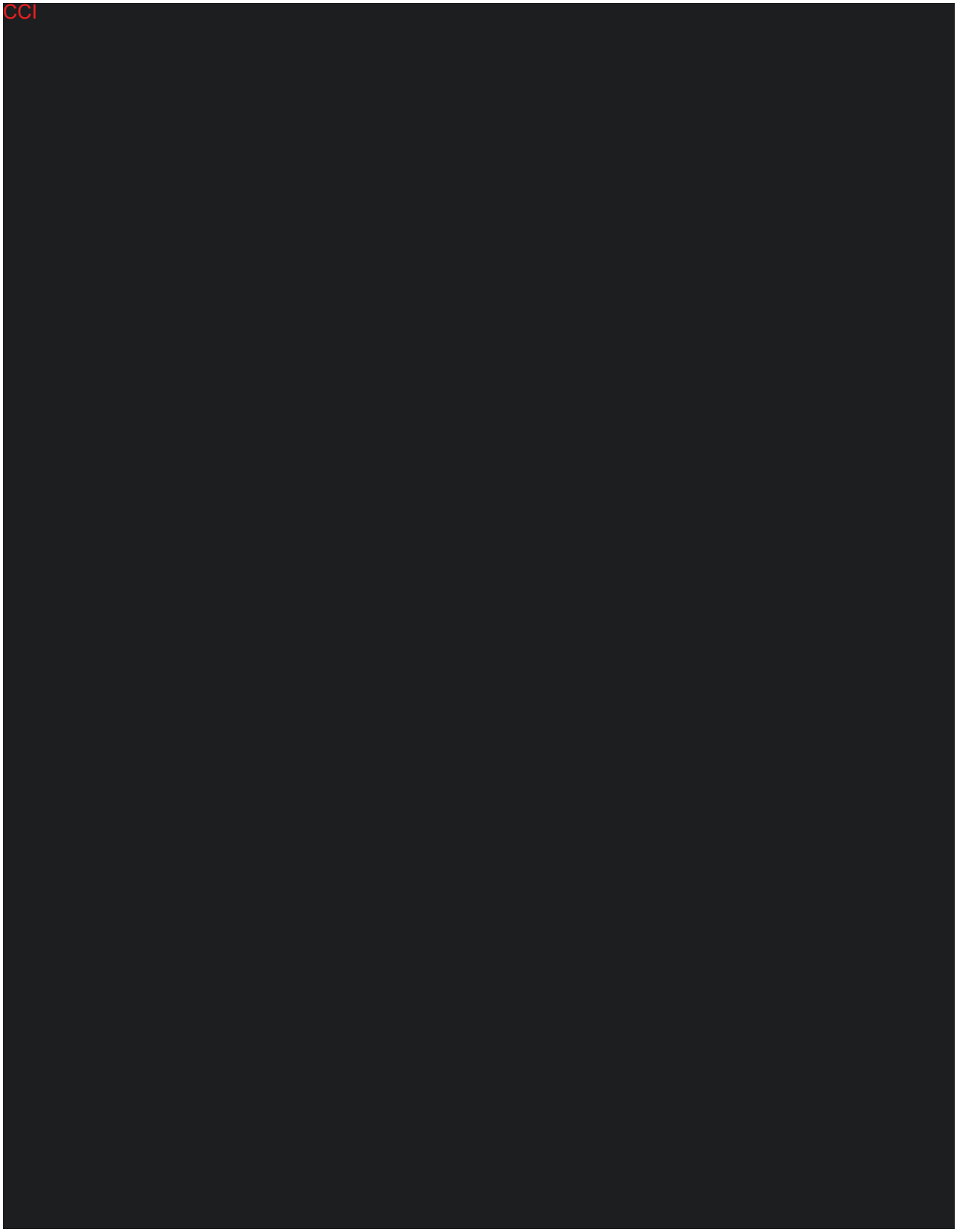

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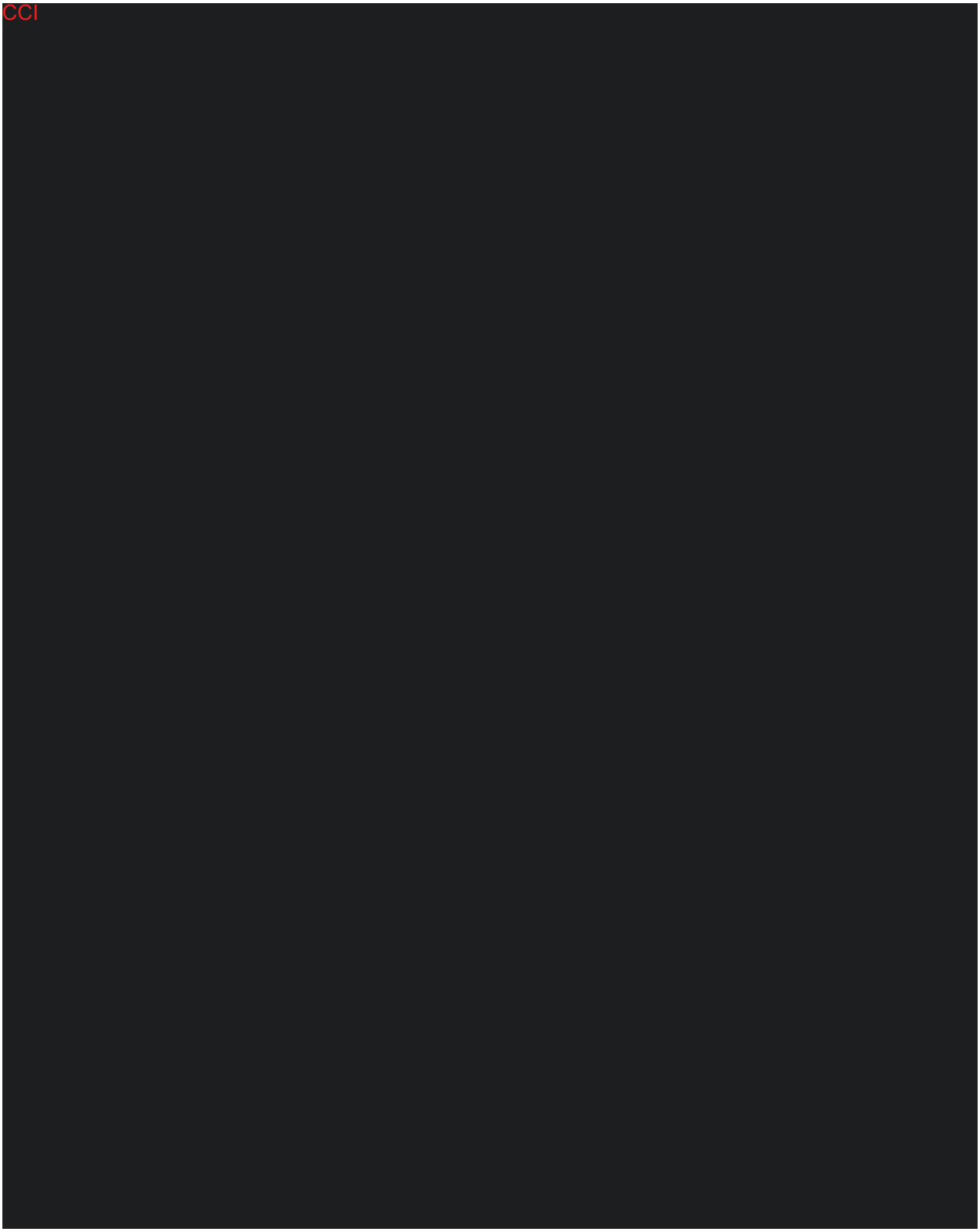

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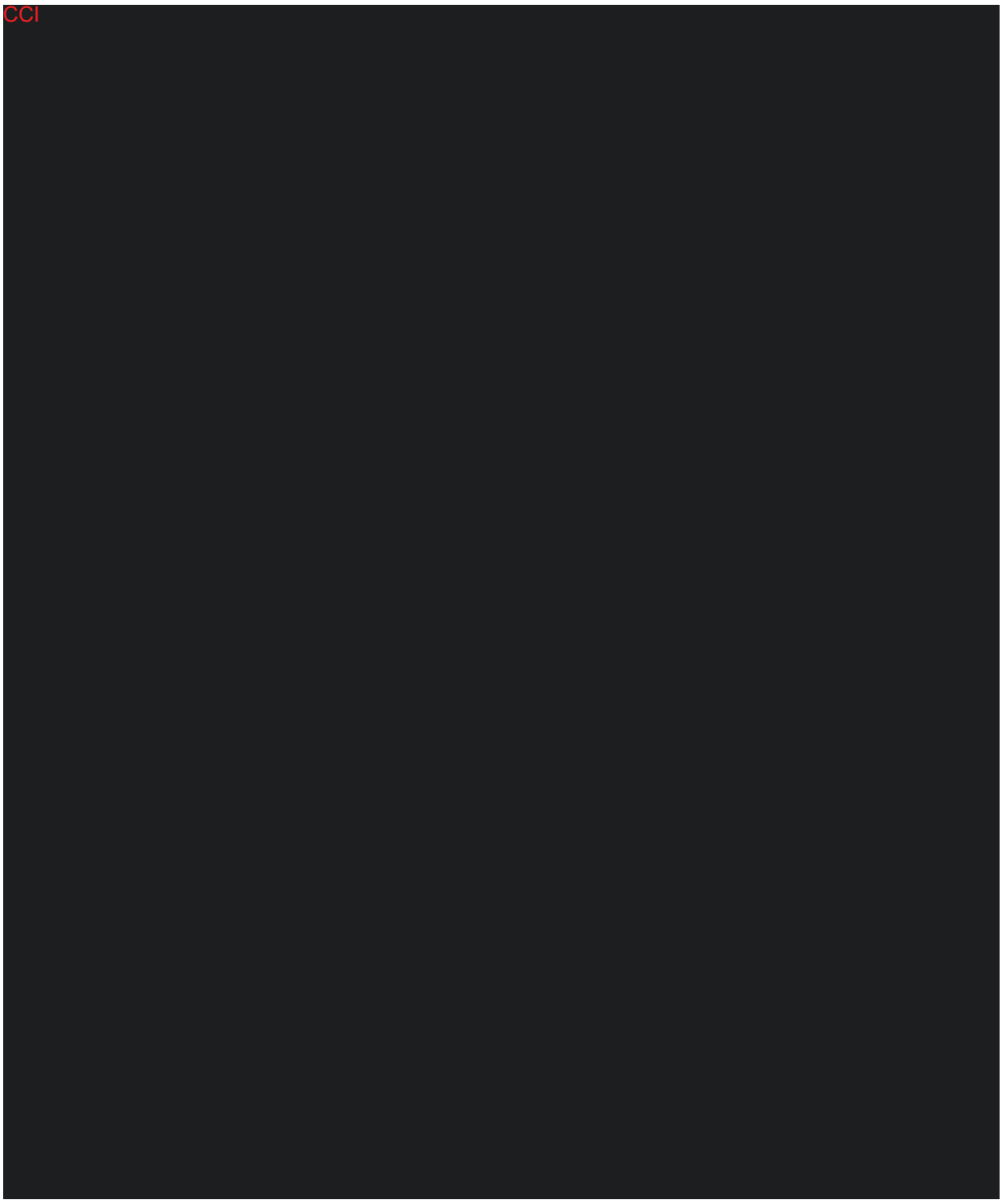

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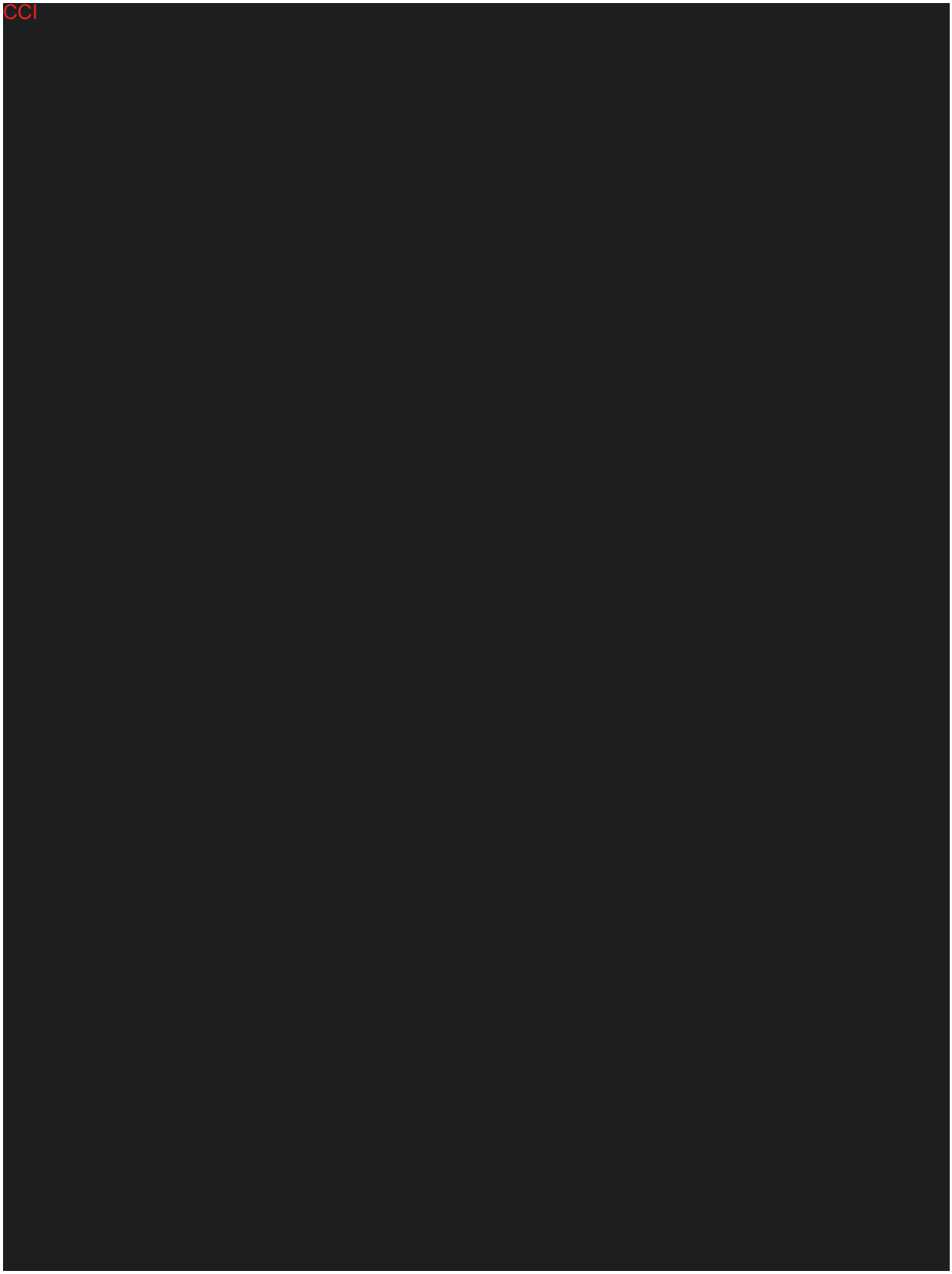

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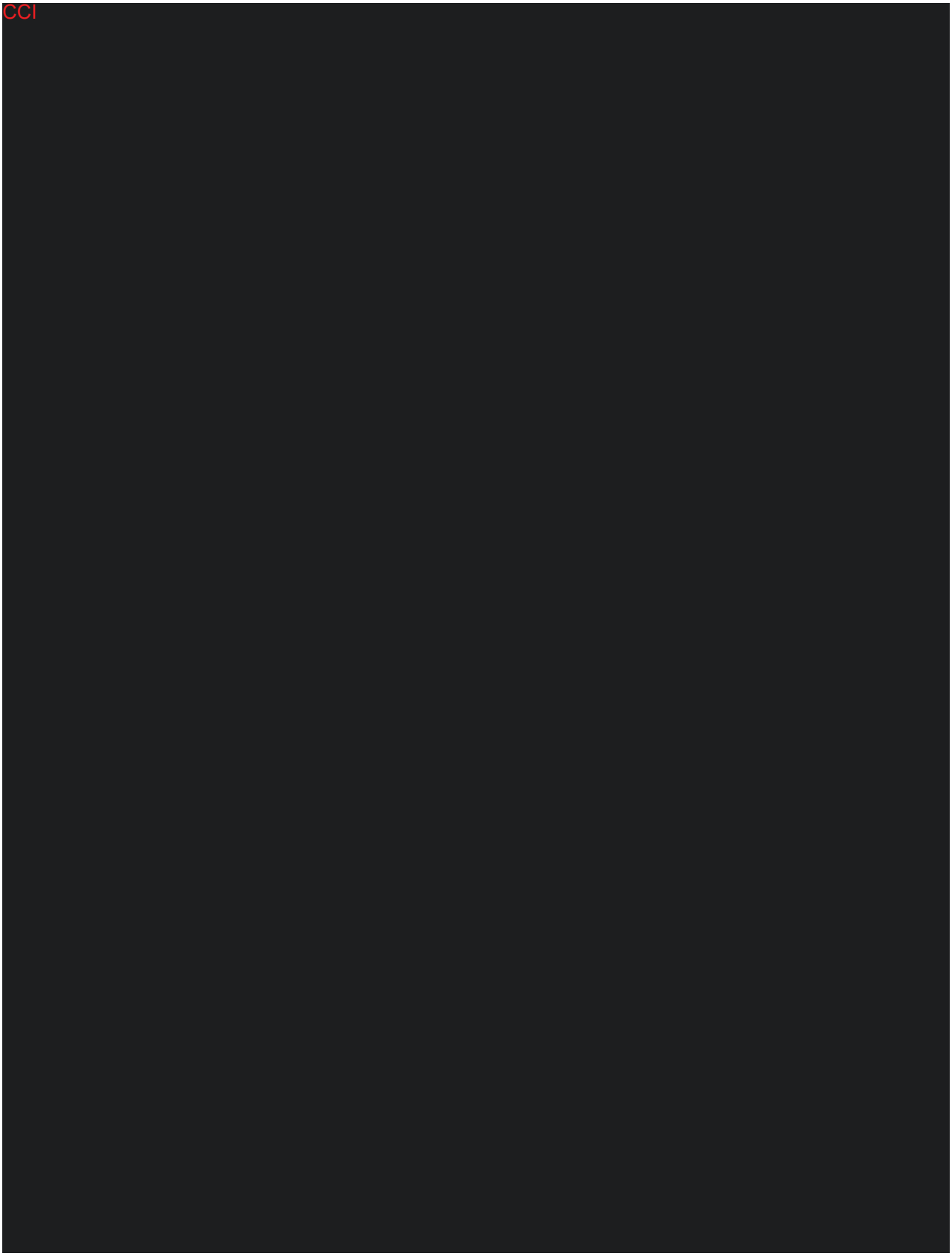

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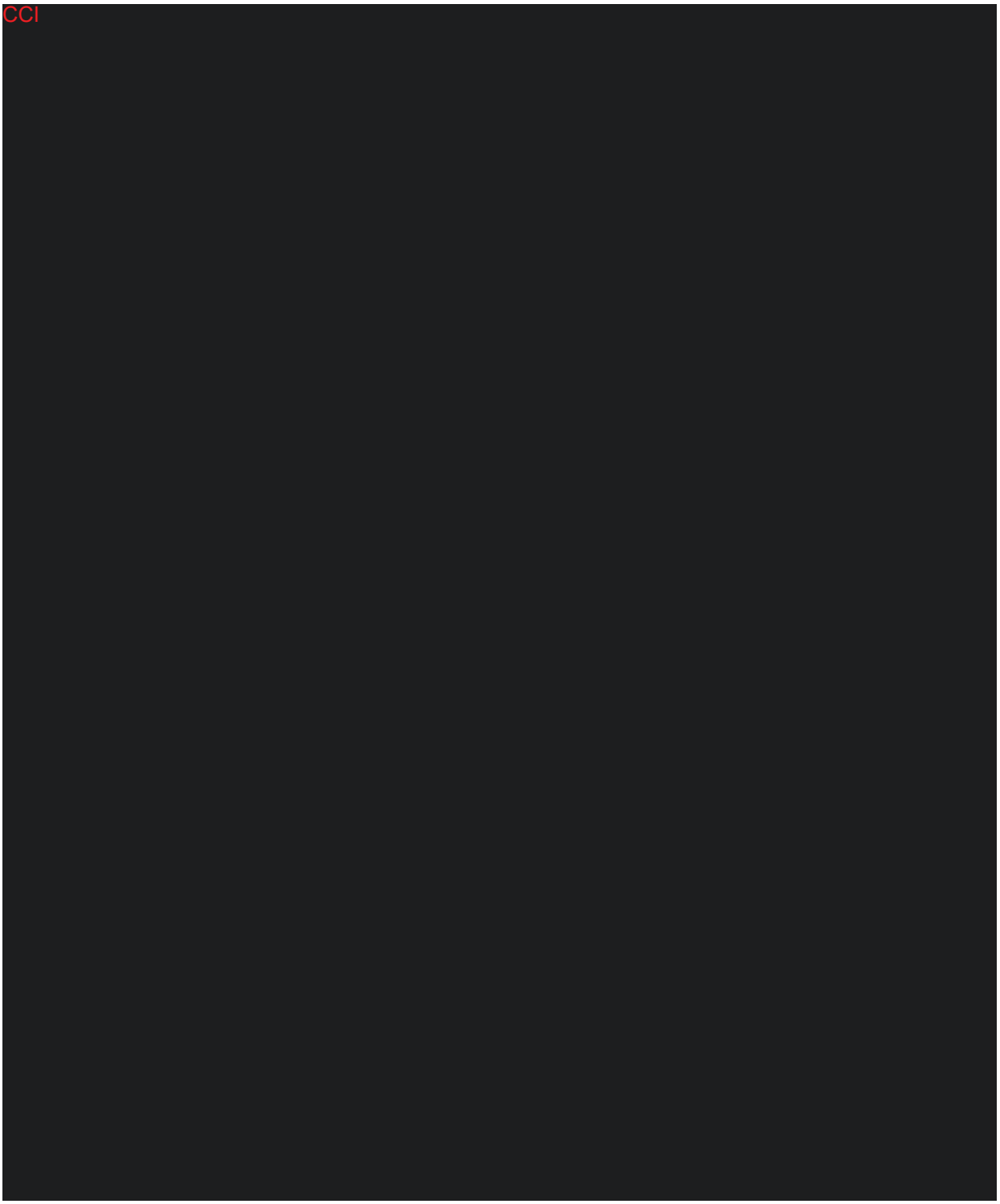

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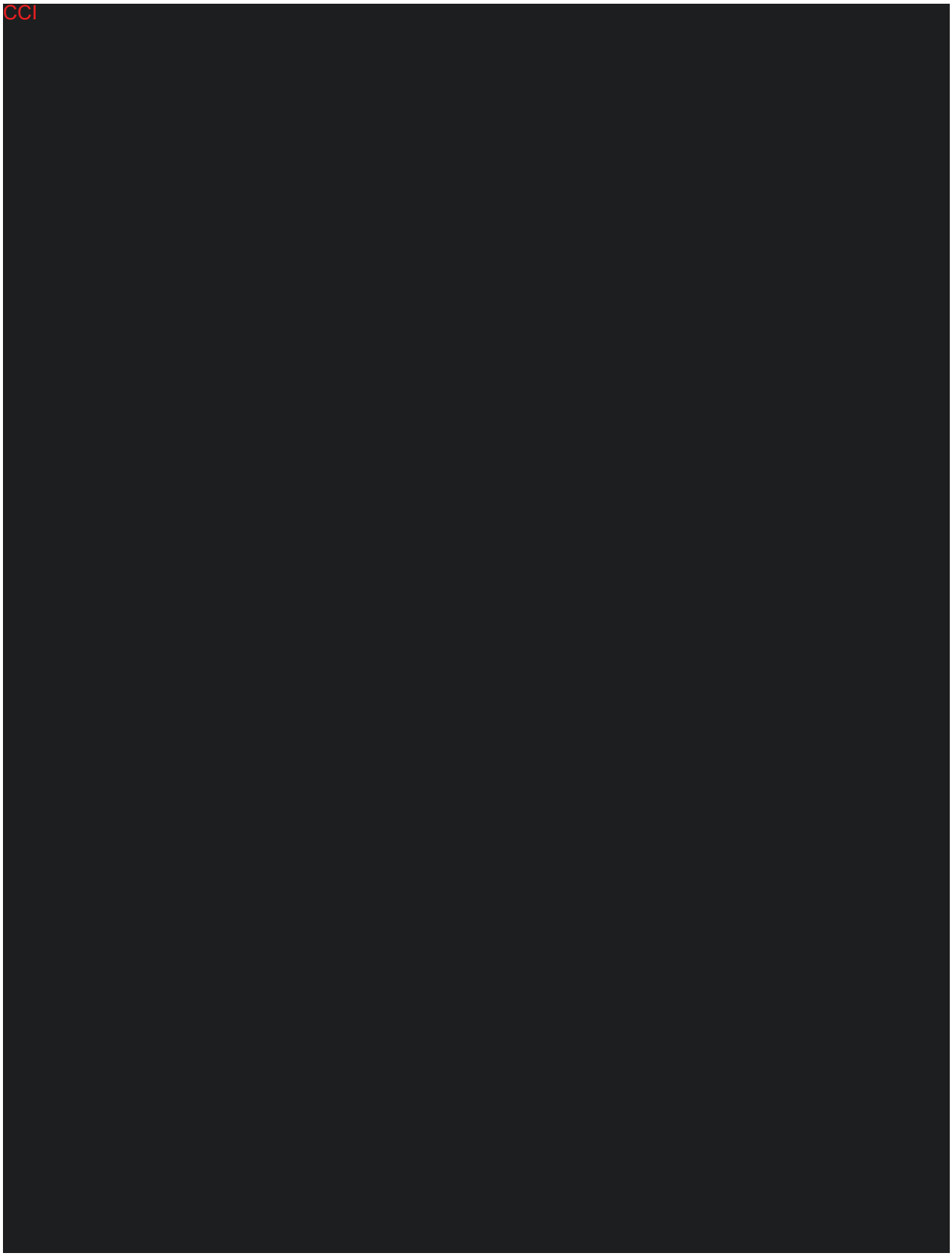

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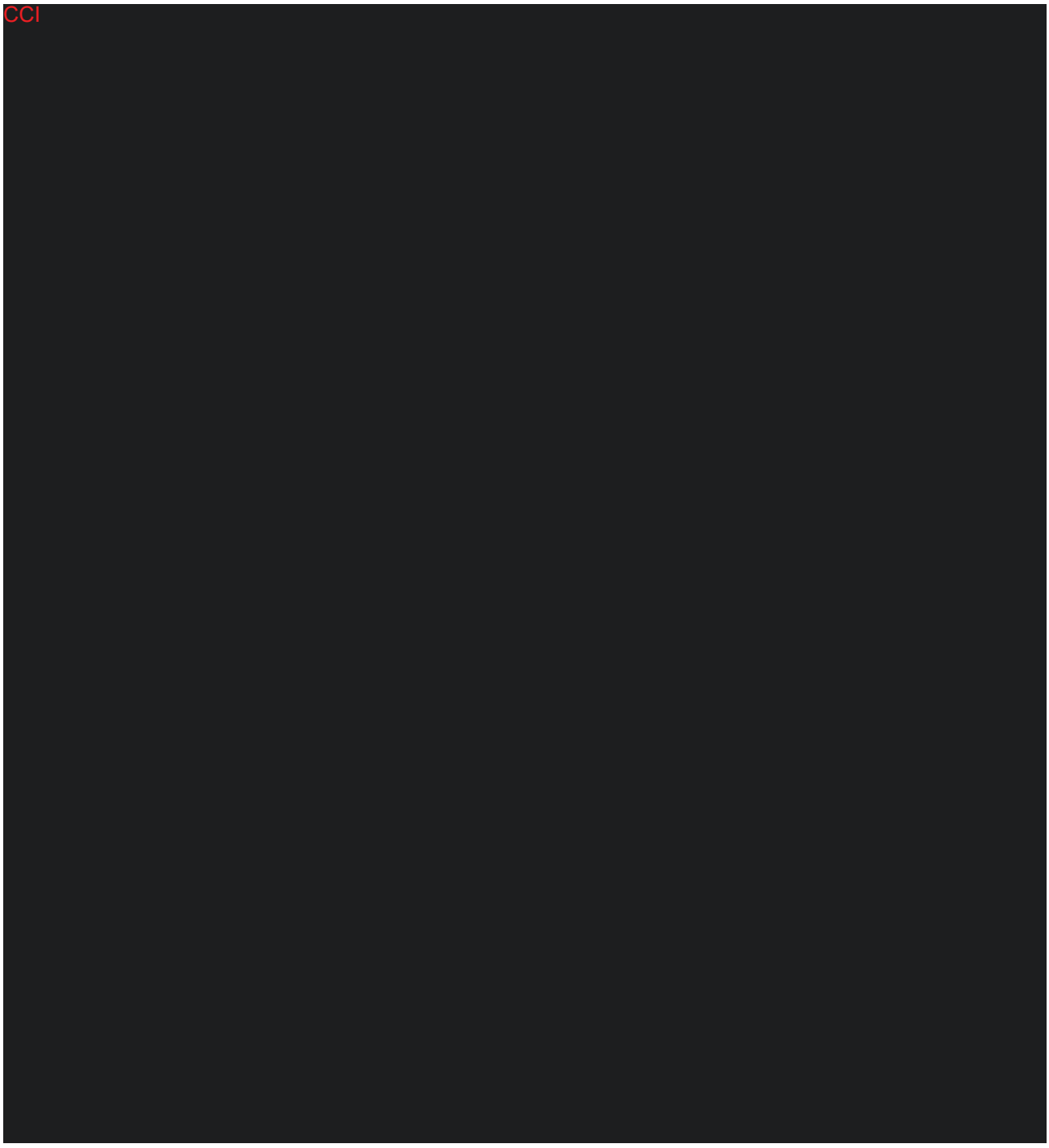

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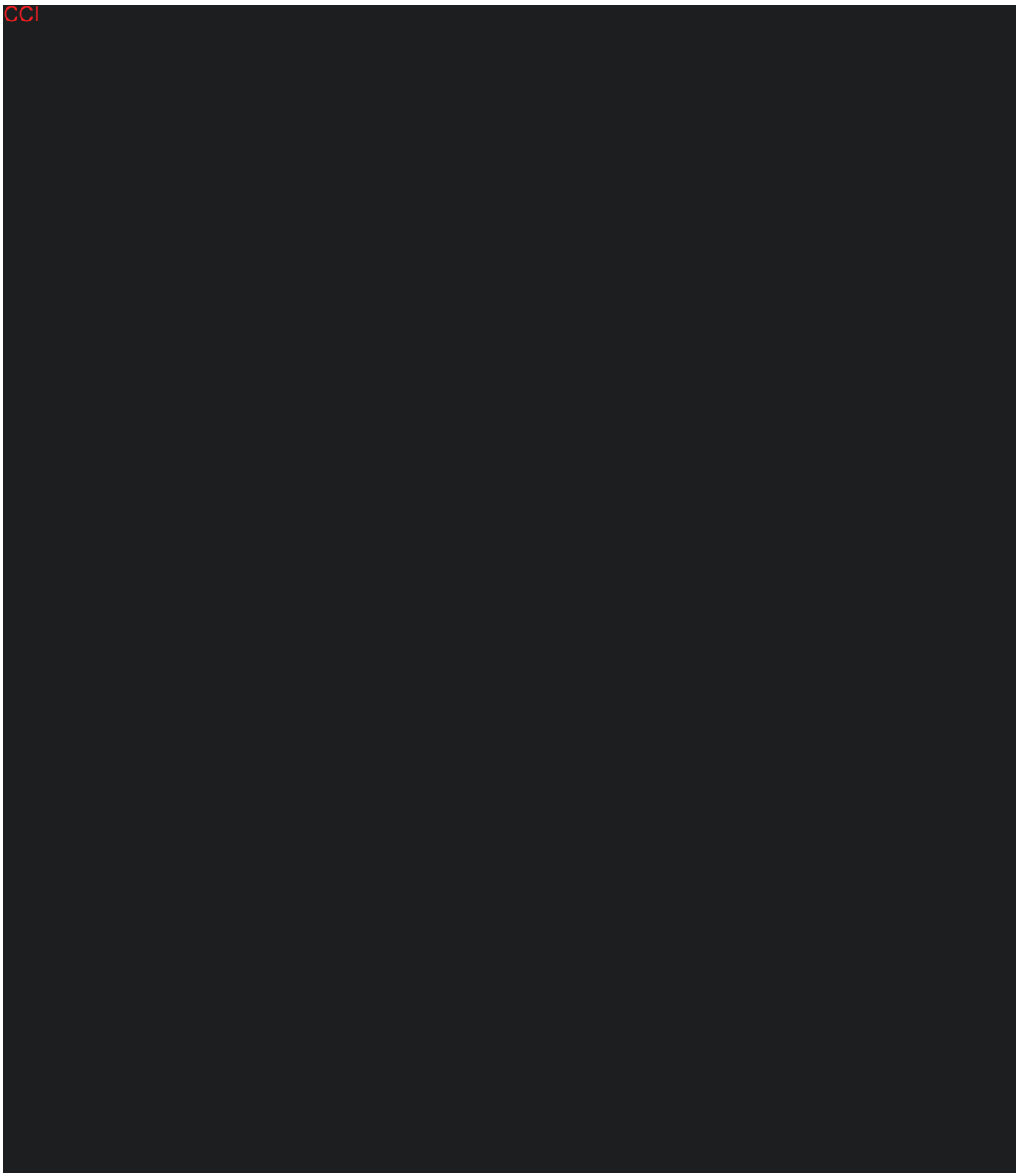

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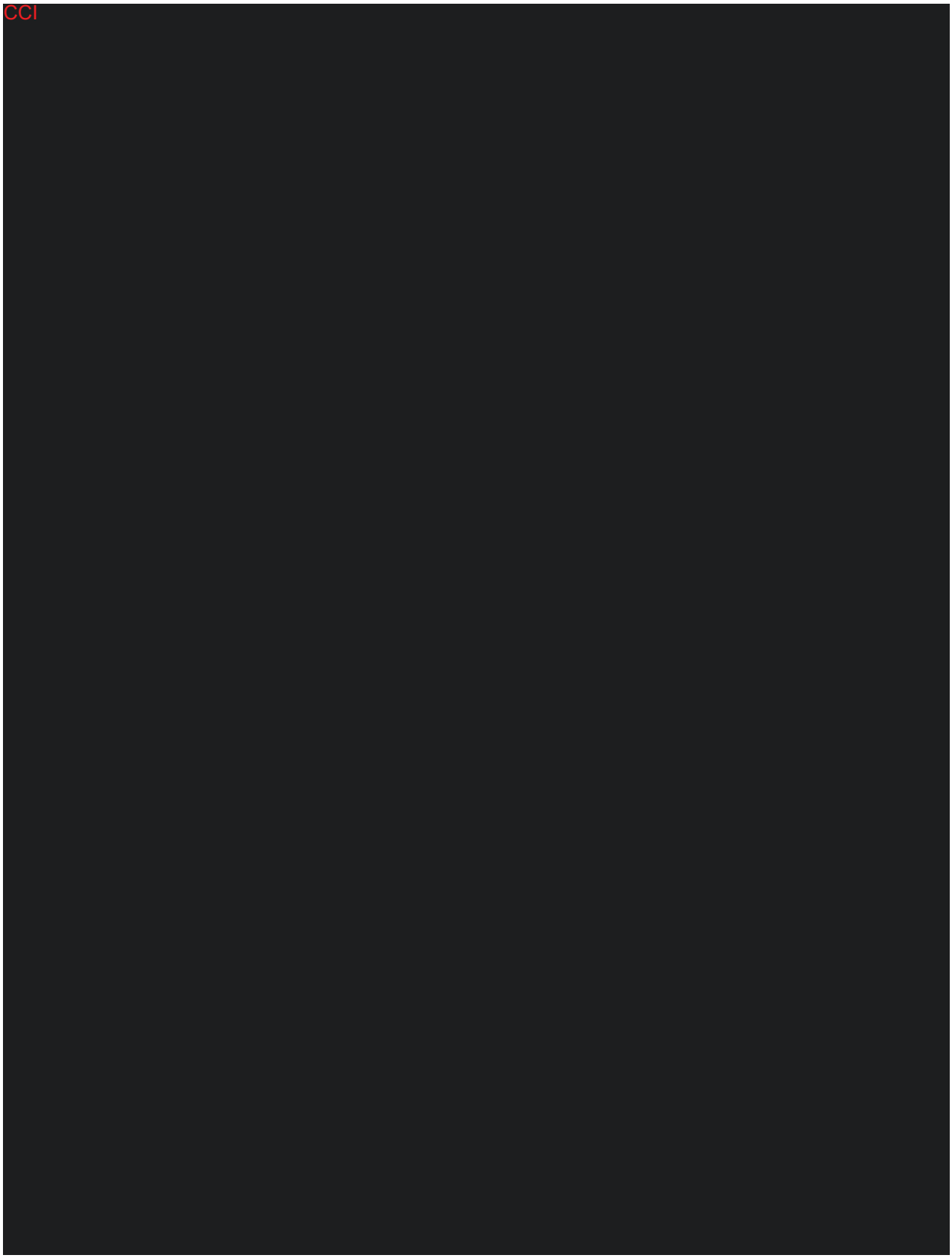

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Signature on Behalf of Sponsor

Study Title: A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A

Protocol Number: CSL627_3001

I have read the Clinical Study Protocol Amendment 5 titled "A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A" and confirm that, to the best of my knowledge, the protocol accurately describes the design and conduct of the study.

PPD

PPD

(Signature)

PPD

Date (DD MMM YYYY)

PPD

(Printed name)

PPD

(Title)

Signature of International Co-ordinating Investigator

Study Title: A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A

Protocol Number: CSL627_3001 **Site Number:** PPD

have read the Clinical Study Protocol Amendment 5 titled "A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A".

By signing this Clinical Study Protocol, I agree to conduct the clinical study, after approval by an Institutional Review Board or Independent Ethics Committee (as appropriate), in accordance with the Clinical Study Protocol, the guideline for Good Clinical Practice (as defined by the International Conference on Harmonisation, ICH GCP) and applicable regulatory requirements.

Changes to the Clinical Study Protocol will only be implemented after written approval is received from CSL Behring and the Institutional Review Board or Independent Ethics Committee (as appropriate) with the exception of medical emergencies.

will ensure that study staff fully understand and follow the Clinical Study Protocol.

PPD

PPD

(Signature) Date (DD MMM YYYY)

PPD

(Printed name) PPD

PPD

(Title)

Signature of Principal Investigator

Study Title: A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A

Protocol Number: CSL627_3001 **Site Number:**

I have read the Clinical Study Protocol Amendment 5 titled "A Phase 3 Open-Label, Multicenter, Extension Study to Assess the Safety and Efficacy of Recombinant Coagulation Factor VIII (rVIII-SingleChain, CSL627) in Subjects with Severe Hemophilia A".

By signing this Clinical Study Protocol, I agree to conduct the clinical study, after approval by an Institutional Review Board or Independent Ethics Committee (as appropriate), in accordance with the Clinical Study Protocol, the standards of Good Clinical Practice (as defined by the International Conference on Harmonisation) and applicable regulatory requirements.

Changes to the Clinical Study Protocol will only be implemented after written approval is received from CSL Behring and the Institutional Review Board or Independent Ethics Committee (as appropriate) with the exception of medical emergencies.

I will ensure that study staff fully understand and follow the Clinical Study Protocol.

_____ _____
(Signature) Date (DD MMM YYYY)

_____
(Printed name)

_____
(Title)

Signature Page

CSL627_3001 - Protocol Amendment - 5 - 26May2020

Signed By	Date (GMT)
PPD	08-Jun-2020 12:54:57
Approved-PPD Approval	
PPD	08-Jun-2020 15:18:59
Approved-Clinical Safety Physician Approval	
PPD	08-Jun-2020 16:59:47
Approved-PPD Approval	

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