Statistical Analysis Plan

CSL Behring GmbH

Protocol: **CSL654_3003**

EudraCT: **2012-005489-37**

IND Number: CCI

Treatment: **Recombinant Fusion Protein Linking Coagulation Factor IX with Albumin (rIX-FP)**

A Phase 3b Open-label, Multicenter, Safety and Efficacy Extension Study of a Recombinant Coagulation Factor IX Albumin Fusion Protein (rIX-FP) in Subjects with Hemophilia B

Authors: PPD

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Contents

Approval Signatures

Signature	PPD		PPD
Print Name:	PPD		
Title	PPD , Biostatistics CCI		

Signature		Date:	
Print Name:	PPD		
Title	PPD , Coagulation Clinical Research & Development CSL		

Signature		Date:	
Print Name:	PPD		
Title	PPD CSL		

Approval Signatures

Signature		Date:	
Print Name:	PPD		
Title	PPD , Biostatistics CCI PPD		

Signature		Date:	PPD
Print Name:			
Title	PPD , Coagulation Clinical Research & Development CSL		

Signature		Date:	
Print Name:	PPD		
Title	PPD CSL		

Approval Signatures

Signature		Date:	
Print Name:	PPD		
Title	PPD , Biostatistics CCI		

Signature		Date:	
Print Name:	PPD		
Title	PPD t, Coagulation Clinical Research & Development CSL		

Signature	PPD	Date:	PPD
Print Name:	PPD		
Title	PPD CSL		

Author Signature

Signature	PPD	Date:	PPD
Print Name:			
Title	PPD CCI		

Abbreviations

ABR	Annualized bleeding rate
AsBR	Annualized spontaneous bleeding rate
AE	Adverse Event
AESI	Adverse event of special interest
AR	Accumulation Ratio
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Concentration-Time Curve
BMI	Body Mass Index
BU	Bethesda Units
CDF	Cumulative Distribution Function
CHO	Chinese Hamster Ovary
CI	Confidence Interval
COVID-19	Coronavirus Disease 2019
CSR	Clinical Study Report
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
eCRF	Electronic Case Report Form
ED	Exposure Day
eDiary	Electronic diary
FDA	Food and Drug Administration
FIX	Coagulation factor IX
GCP	Good Clinical Practice
ICH	International Council for Harmonisation
IR	Incremental Recovery
IU	International unit
IV	Intravenous
LL	Local Laboratory
MAP	Modeling Analysis Plan
MedDRA	Medical Dictionary for Regulatory Activities
NCA	Noncompartmental Analysis
OS	One-stage Clotting
PCS	Potentially Clinically Significant
pdFIX	Plasma-derived Factor IX
PK	Pharmacokinetic
PP	Per-Protocol
PT	Preferred Term
PTP	Previously Treated Patient
PUP	Previously Untreated Patient
CCI	[redacted]
rFIX	Recombinant FIX
rIX-FP	Recombinant Coagulation Factor IX Albumin Fusion Protein
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SC	Subcutaneous
SD	Standard deviation
SMQ	Standardized MedDRA Query
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TSS	Transformed Scale Score
WFH	World Federation of Hemophilia
WHO	World Health Organization

1 Introduction

This document presents the statistical analysis plan (SAP) for CSL Behring GmbH, Protocol No. CSL654_3003: A Phase 3b Open-label, Multicenter, Safety and Efficacy Extension Study of a Recombinant Coagulation Factor IX Albumin Fusion Protein (rIX-FP) in Subjects with Hemophilia B.

This analysis plan is based on the protocol amendment 6 dated 03 February 2020, final electronic case report form (eCRF) dated 17 May 2017, and the SAP version 3 dated 20 April 2018. This SAP contains all analyses for the previously treated patients (PTPs), and the updated contents for the previously untreated patients (PUPs).

In the event that the statistical methods specified in this SAP differ from those specified in the protocol, the statistical methods specified in the SAP will supersede the statistical methods described in the protocol.

2 Study Objectives

- Main Study
  - Primary Objective
    - The primary objective of this study is to evaluate the safety of rIX-FP as measured by new cases of inhibitors against FIX in subjects, including PUPs, with severe hemophilia B. The PK parameters in PUPs will also be collected.
  - Primary Endpoint(s)
    - The total number of subjects who develop inhibitors against FIX during the approximately 5-year participation in this study.
    - PK parameter of incremental recovery (IU/dL per IU/kg) of 50 IU/kg rIX-FP (Arm 4 only)
  - Secondary Objective(s)

    The secondary objectives of the study are:
    - To evaluate the efficacy of rIX-FP routine prophylaxis when administered at various treatment intervals.
    - To compare the efficacy of rIX-FP routine prophylaxis between 2 different treatment intervals and versus on-demand treatment.
    - To further evaluate the safety of rIX-FP.
    - To evaluate the efficacy of treatment for bleeding episodes in PUPs.
  - Secondary Endpoint(s)
    - Annualized bleeding rate (ABR) for spontaneous treated and total treated bleeds for each assigned treatment interval (7 days, 10 days, 14 days, and 21 days).
    - Comparison of the annualized spontaneous bleeding rate (AsBR) and ABR between:

  - 14-day routine prophylactic treatment in this study compared with on-demand only treatment from Study CSL654_3001 Arm 2.
  - 7-day prophylaxis regimen with the 14-day prophylaxis regimen.
  - 7-day prophylaxis regimen with the extended prophylaxis regimen (10-day or 14-day).
- Total number of exposure days of rIX-FP
- Consumption of rIX-FP during routine prophylaxis expressed as IU/kg per month per subject.
- Incidence of (AEs) and related AEs to rIX-FP over the course of the study.
- Hemostatic response to treatment of bleeding events with rIX-FP in PUPs as assessed by the investigator based on a 4-point scale (Arm 4 only).

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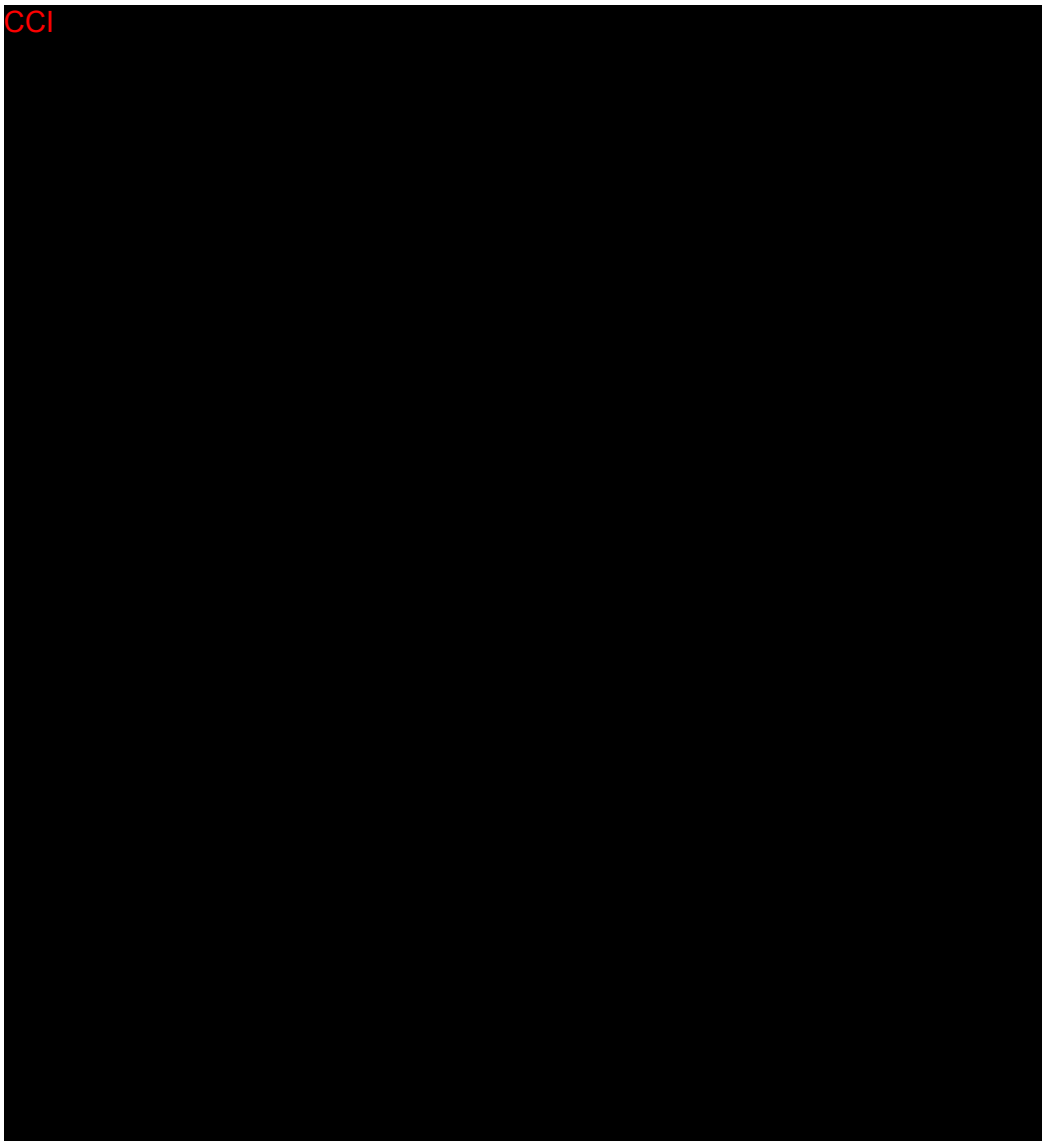


3 Study Design

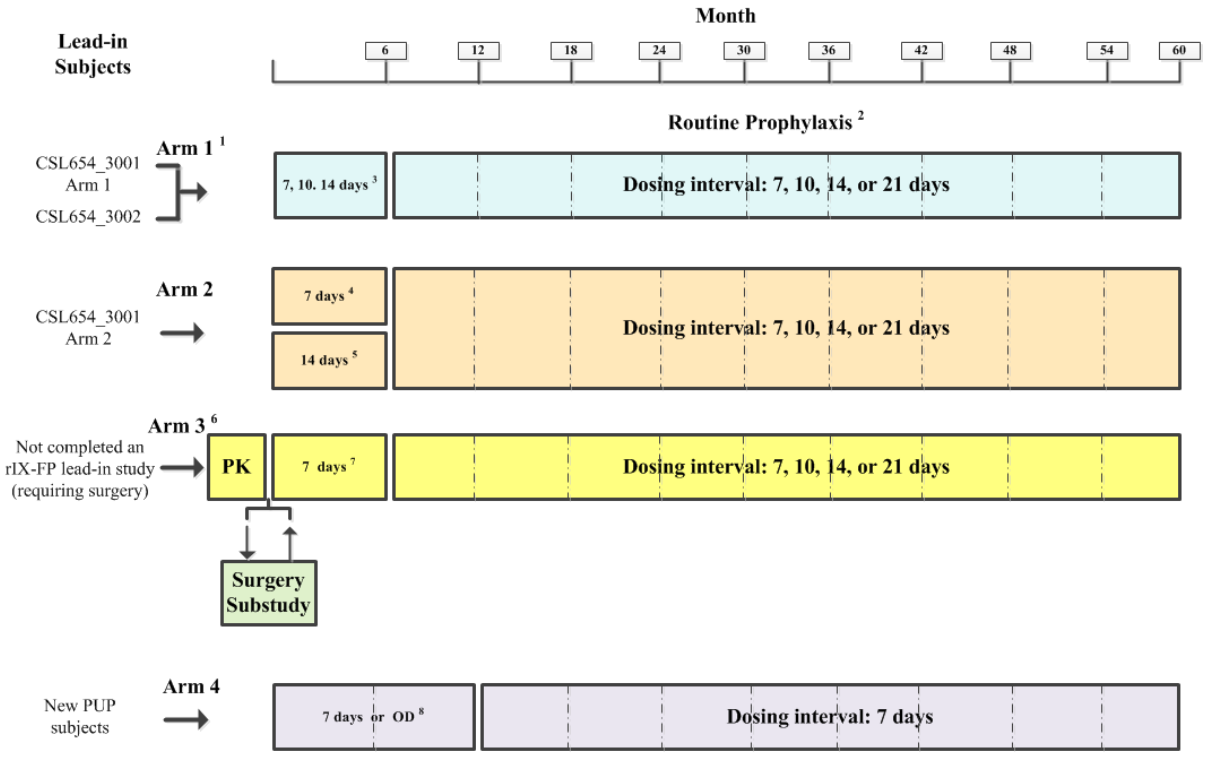
3.1 Discussion of Study Design

This is a prospective, open-label, multicenter, non-randomized, phase 3b study to investigate the long-term safety and efficacy of recombinant coagulation factor IX albumin fusion protein (rIX-FP) for the routine prophylaxis and on-demand treatment of bleeding events in subjects with severe hemophilia B. Subjects will be eligible to enter the study if they are PUPs and meet the eligibility criteria (i.e. Arm 4).

Subjects who completed studies CSL654_3001, CSL654_3002, or any other CSL sponsored rIX-FP (CSL654) studies (i.e. Arm 1 and Arm 2) and meet the eligibility criteria, or subjects who have not previously completed a CSL sponsored rIX-FP lead-in study and meet the eligibility criteria (i.e. Arm 3) have already been analyzed in the clinical study report (CSR) dated 14 March 2019. These subjects will not be analyzed in this PUP study.

The study overview is provided in Figure 1. A complete overview of the study design and rationale is provided in Protocol CSL654_3003, Section 3.1.

Figure 1. CSL654_3003 Study Overview



3.2 Planned Study Duration

For subjects in Arms 1, 2, and 3, the duration of the study for an individual subject is expected to be approximately 5 years or the time it takes to achieve a total of 100 exposure days (EDs) during enrollment in any CSL-sponsored rIX-FP studies. For subjects in Arm 4, the duration of the study is expected to be approximately 3 years or the time it takes to achieve a total of 50 EDs.

The overall study duration (i.e., first subject's first visit to the last subject's end of study visit) is expected to be approximately 5 years. The study may end after 100 EDs of rIX-FP have been achieved in ≥50 subjects overall for rIX-FP (i.e., treatment received across all studies).

The study may be stopped at a specific study site if at least 1 of the following occurs:

- All subjects have completed the 5-year study period, or
- ≥ 50 subjects overall have achieved a total of 100 EDs, or
- Regulatory approval of CSL654 is granted and rIX-FP becomes commercially available in the respective country

3.3 Planned Sample Size

The choice of sample size for this study is not based on statistical power considerations. This study will enroll approximately 96 male subjects, including all eligible subjects from rIX-FP lead-in studies and approximately 10 subjects requiring major non-emergency surgery who

have not previously received treatment with rIX-FP and at least 13 PUPs. The target is at least 50 subjects completing 100 EDs during enrollment in all CSL-sponsored rIX-FP studies, as per the European Medicines Agency guideline (EMA/CHMP/BPWP/144552/2009 rev 1).

3.4 Study Arms

This study will include the following study arms:

Previously Treated Patients:

Arm 1: Subjects who have completed Study CSL654_3001 Arm 1, Study CSL654_3002, or any other CSL-sponsored rIX-FP (CSL654) lead-in study will administer rIX-FP once every 7, 10 or 14 days.

Arm 2: Subjects who were enrolled in Study CSL654_3001 Arm 2 and have <26 weeks of experience with rIX-FP will administer rIX-FP once every 7 days. Subjects who have ≥26 weeks of experience with weekly prophylaxis rIX-FP will administer rIX-FP once every 14 days.

Arm 3: Subjects who have not previously completed a CSL-sponsored rIX-FP lead-in study and who are scheduled to have a major non-emergency surgery within approximately 8 weeks from receiving the first rIX-FP injection (i.e., during the pharmacokinetics [PK] evaluation period) will administer rIX-FP once every 7 days until the month-6 visit.

After the month-6 visit, all subjects (Arms 1, 2, and 3) will administer rIX-FP once every 7, 10, 14 days, 21 days, or 3 times per month for approximately 30 months.

Note: All subjects in Arm 3 will undergo an initial rIX-FP (100 IU/kg) PK evaluation period and complete a surgery substudy before having the option to start routine prophylaxis. In addition, a subcutaneous (SC) substudy will evaluate the safety and PK of SC administration of rIX-FP in adults with severe hemophilia B who are enrolled in the main CSL654_3003 study.

Previously Untreated Patients:

Arm 4: PUPs with severe Hemophilia B (coagulation factor IX (FIX) activity ≤2%) who have never been treated with FIX clotting factor products (except previous exposure to blood components) will be enrolled. Subjects in Arm 4 will administer rIX-FP as weekly prophylaxis and/or on-demand during the first 12 months, and then continue on weekly routine prophylaxis.

This SAP is focusing on the statistical analyses on patients in Arm 4.

3.5 Schedule of Assessments

The Schedule of Assessments for the treatment period (Day 1 to End of Study) during routine prophylaxis for Arms 1, 2, and 3 is provided in Protocol CSL654_3003 Table A, and in Figure 2 of this SAP. The Schedule of Assessments during the PK Period is provided in Protocol CSL654_3003 Table B, and Figure 3 of this SAP. Additional details for subjects in Arm 3 are provided in Figure 4. The Schedule of Assessments for subjects in Arm 4 is provided in Protocol CSL654_3003 Table D, and Figure 5 in this SAP. The Schedule of

Assessments for major surgery or minor surgery are provided in Protocol CSL654_3003 Appendix 1, and the Schedule of Assessments for the SC substudy is provided in Protocol CSL654_3003 Appendix 2.

Figure 2. Schedule of Assessments: Treatment Period (Arms 1, 2, and 3)

Assessments	Day 1 [B] Arm 1 & 2	Month [A] 3 [C]	6 [L]	9 [C]	12	18, 30, 42, 54	24, 36, 48, 60	50 EDs [D]	End of Study
Informed consent	X								
Demographics, lead-in study data	X								
Eligibility assessment	X								
Social and physical activity	X		X		X	X	X		X
Relevant medical history [E]	X								
Physical examination	X				X		X		X
Vital signs	X				X		X		X
Body weight and height [F]	X	X	X	X	X	X	X		
Blood biochemistry and hematology (LL)	X				X				X
Inhibitors against FIX (CL)	X		X		X	X	X	X	X
Ab against rIX-FP & CHO cell proteins (CL) [G]	X				X		X	X	X
Plasma FIX activity (CL)	X[M]		X		X	X	X		
Plasma FIX activity (LL)			X		X				
Retain blood sample [H]									X
Dispense subject eDiary	X								
Review subject eDiary		X	X	X	X	X	X		X
Assess treatment efficacy for a major bleed		X	X	X	X	X	X		X
Review treatment regimen [I]			X		X	X	X		
Hemo-Sat questionnaire [J]					X				
CCI					X				
Retrieve eDiary									X
Adverse events	<----------On an ongoing basis---------->							X	X[K]
Concomitant therapy	<----------On an ongoing basis---------->							X	X

Ab = antibody; CHO = Chinese hamster ovary; CL = central laboratory; ED = exposure day; eDiary = electronic diary; FIX = coagulation factor IX; LL = local laboratory; CCI; rIX-FP = recombinant coagulation factor IX albumin fusion protein.

Notes to the schedule of assessments:

A: Time window: ± 2 weeks. All visits should occur within 2 calendar days before the next scheduled prophylactic administration of rIX-FP. A month is 28 days.

B: Day 1 assessments to be completed only by subjects in Arms 1 or 2. Data that will be transferred from the end of study visit or earlier visit (where relevant) of the lead-in study include demographics, rIX-FP EDs, age cohort in lead-in study, arm in lead-in study, social and physical activity, laboratory assessments (serum biochemistry, hematology), vital signs, physical examination, body height and weight, and antibody assessments (inhibitors against FIX, antibodies against rIX-FP & CHO cell-derived proteins), plasma FIX activity (in subjects from CSL654_3001 study only), adverse events, and concomitant therapy.

C: For subjects from lead-in Study CSL654_3001 Arm 1, the month 3 and month 9 visits may be conducted via telephone, if the investigator deems that there is no safety risk to the subject. For subjects from lead-in Study CSL654_3001 and CSL654_3002, the month 9 visit may be conducted via telephone, if the investigator deems that there is no safety risk to the subject. The month 3 visit may be omitted for subjects in Arm 2 who have attended a follow-up after visit in the previous 3 months to transition from a 7-day rIX-FP treatment interval to a 14-day rIX-FP treatment interval.

D: Visit to occur within a month after a subject has experienced a total of 50 rIX-FP EDs in this extension study. If the 50 EDs visit is to occur within 3 months of a follow-up visit, the scheduled follow-up visit may be omitted by conducting all follow-up assessments, in addition to assessments required for the 50 EDs visit, during the 50 EDs visit.

E: Record genotype and current joint score including scoring system and date of the assessment.

F: Height to be recorded on day 1 and thereafter at annual intervals only (ie, month 12, 24, etc). Height measurements on the month 12, 24, 36, and 48 visits are optional for subjects ≥ 18 years of age. Measurement of body weight may be omitted at month 3 and month 9 if the visit is conducted via telephone.

G: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between plasma-derived FIX, recombinant FIX and albumin antibodies.

H: Retain samples will be collected for potential serology testing at a later date.

I: Investigator to review treatment efficacy and change treatment regimen (dose and / or treatment interval), if necessary.

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K: 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 end of study visit. However, adverse events ongoing at the end of study visit will be followed until resolution or 30 days after the final administration of rIX-FP during this study, whichever is sooner.

L: Arm 2 Subjects should attend an unscheduled visit and complete assessments as specified for the 6-month visit if they are to switch to a 14-day treatment interval before completion of the 6-month period. Under such circumstances, the 3-month visit may be omitted.

M: Sample collected in subjects coming from Study CSL654_3002 after consenting for CSL654_3003.

Figure 3. Schedule of Assessments: Pharmacokinetic Period

Assessments	Before injection	50 IU/kg (Arm 4) or 100 IU/kg rIX-FP PK Visit [A]					
		0 hour (injection)	30 min	72 hours [B] (3 days)	168 hours [C] (7 days)	336 hours [D] (14 days)	504 hours [D] (21 days)
Time Window	< 12 hours before injection		± 5 min	± 24 hrs	± 24 hrs	± 24 hrs	± 24 hrs
Weight and height	X						
Plasma FIX level (central)	X		X	X	X	X	X
Retain blood sample [E]	X						
Administration of rIX-FP		X					
Dispense subject eDiary [F]		X					
Training of self-administration [F]		X	X	X	X		
Adverse events		<----------On an ongoing basis---------->					
Concomitant therapy		<----------On an ongoing basis---------->					

eDiary = electronic diary; FIX = coagulation factor IX; PK = pharmacokinetic; rIX-FP = recombinant coagulation factor IX albumin fusion protein.

A: 100 IU/kg PK completed only by subjects in Arm 3 (no subjects in France may enroll in Arm 3) or subjects ≥18 years who have completed at least 6 months of treatment and planning to begin routine prophylaxis treatment using a 21-day treatment interval for the first time. Subjects in France may not take part in the 21 day treatment regimen. The PK evaluation should be performed after a washout period of at least either 4 days for a current marketed FIX product (ie, subjects in Arm 3 (no subjects in France may enroll in Arm 3) or 14 days for rIX-FP (subjects currently being treated with rIX-FP). 50 IU/kg PK completed by subjects in Arm 4 (see Table D for assessments prior to rIX-FP injection). The investigator may also choose to complete a PK assessment of 50, 75 or 100 IU/kg (as appropriate) rIX-FP with selected time points before starting surgical prophylaxis with rIX-FP, at the investigator's discretion or CSL's request, or in the event of (but not limited to) poor efficacy or suspicion of inhibitor development. Samples may be tested in the local laboratory in addition to the central laboratory. Subjects in France may be discontinued from receiving the study product and participating in any further study procedures if the subject is confirmed to have developed an inhibitor against FIX with a titer > 5 BU/mL.

B: Optional time point for subjects planning to be switched to a 21-day treatment interval, and Arm 4.

C: Optional time point for Arm 4 subjects.

D: Optional time point for subjects in Arm 3 before major surgery (No subjects in France may enroll in Arm 3). Not done for Arm 4.

E: Retain samples will be collected before the first injection of rIX-FP (subjects in Arm 3 only, excludes subjects in France) for potential serology testing at a later date.

F: Subjects in Arm 3 only (subjects in France may not enroll in Arm 3). Before completion of the PK evaluation period (at a time point determined by the investigator), the eDiary should be dispensed and subjects should be trained on injection technique, dosing regimen, and use of the eDiary.

Figure 4. Schedule of Assessments: Subjects in Arm 3 (No Subjects in France May Enroll in Arm 3)

	Screening	PK Period	Before surgery	Surgery Substudy	Day 1 [A] Arm 3	Treatment Period
Time window	-1 to -28 days		7 to 56 days after PK injection	Surgery day to 28 ± 7 days after surgery		
Assessments						
Informed consent	X					
Demographics	X					
Eligibility assessment	X					
Social and physical activity	X					
Relevant medical and treatment history [B]	X					
Physical examination	X					
Vital signs	X					
Body weight and height	X				X [C]	
Blood biochemistry and hematology (LL)	X					
Inhibitors against FIX (CL)	X					
Ab against rIX-FP & CHO cell proteins (CL) [D]	X					
Plasma FIX activity (CL and LL)	X					
Begin prophylaxis treatment with rIX-FP					X [E]	
Adverse events	X		X		X	
Prior / concomitant therapy	X		X		X	
		Complete Schedule of Assessments: Pharmacokinetic Period (Table B)		Complete Schedule of Assessments: Surgery Substudy – Major Surgery		Complete Schedule of Assessments: Treatment Period (Table A)

Ab = antibody; CHO = Chinese hamster ovary; CL = central laboratory; eDiary = electronic diary; FIX = coagulation factor IX; LL = local laboratory; PK = pharmacokinetics; rIX-FP = recombinant coagulation factor IX albumin fusion protein.

Notes to the schedule of assessments:

A: The Day 1 visit should occur after completion of the surgery substudy and should occur at the same time as the End of Surgery Substudy visit.

B: Record history of hemophilia B including genotype and current joint score (including scoring system and date of the assessment), and treatment history with a marketed FIX.

C: Body weight only.

D: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between plasma-derived FIX, recombinant FIX and albumin antibodies.

E: Subjects in Arm 3 (**No subjects in France may enroll in Arm 3**) who require physical therapy (ie, rehabilitation after surgery), may administer rIX-FP as preventative treatment (no more than once per week) in addition to the administration of rIX-FP as routine prophylaxis. Preventative treatment before physical therapy is permitted only during the first 3 months of the treatment period.

Figure 5. Schedule of Assessments: Subjects in Arm 4

Assessments	Screen[B] (up to 28 days)	(PK[A]) Prior dose	(PK[A]) 30±5 mins	(PK[A]) Day 3	(PK[A]) Day 7	Months[I] (±1 week) 1	2	3	4	5	6	7	8	9	10	11	12	Months (±2 week) 15	18	21	24	27	30	33	50 EDs	EoS[L]
Informed consent	X																									
Demographics	X																									
Eligibility assessment	X																									
Relevant medical history	X																									
Physical examination	X					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs[C]	X					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Body weight and height	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Biochemistry&hematology[D]	X[E]																X				X					X
Inhibitors against FIX (CL)	X[E]					X	X	X	X	X	X			X			X		X		X		X		X	X
Ab to rIX-FP/CHO (CL)[F]	X[E]										X						X				X				X	X
Plasma FIX activity (CL)[H]		X	X	X	X	X	X	X			X						X				X					X
Plasma FIX activity (LL)	X[J]																									
Retain blood sample[G]		X																								X
Dispense subject eDiary[K]																	X									
Review subject eDiary						<---------- On an ongoing basis during eDiary usage ---------->																				X
Review treatment efficacy						<---------- On an ongoing basis ---------->																				X
Adverse events	<---------- On an ongoing basis ---------->																									
Concomitant therapy	<---------- On an ongoing basis ---------->																									

Ab = antibody; CHO = Chinese hamster ovary; CL = central laboratory; eDiary = electronic diary; FIX = coagulation factor IX; LL = local laboratory; PK = pharmacokinetics; rIX-FP = recombinant coagulation factor IX albumin fusion protein; EoS=End of Study.
Subjects will be treated in the study center or medical facility under medical supervision during the initial 10 to 20 treatment with rIX-FP. The first 2 infusions must be monitored on-site for at least 3 hours.

A: PK can start at Day 1 as the first dose or/and during the study after ≥7 days washout from the previous rIX-FP dose, when the subject is in non-bleeding state. See Table D for PK sampling time points. (Day 1 is the day subject receives his first dose with rIX-FP)
B: Tests that require blood sample for screening period (from ICF day to Day 1 prior to dose) can be divided to multiple days or combined with Day 1 tests. The screening day can be the same day for first dose of rIX-FP (either on-demand, prophylaxis or PK dose).
C: Vital signs include blood pressure, temperature and heart rate.
D: Includes liver and renal function test (Albumin, Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Bilirubin, Direct Bilirubin, Protein, Blood Urea Nitrogen or Urea, and Creatinine).
E: Documented biochemistry & hematology results from 6 months prior to screening are acceptable; otherwise, samples may be collected during the screening period or at Day 1. Samples for antibodies/inhibitor must be collected during the screening period or prior to first dose of rIX-FP.
F: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between pdFIX, rFIX and albumin antibodies.
G: Retain samples will be collected prior to the first dose of rIX-FP for potential serology testing at a later date.
H: Optional samples at Day 3 or Day 7 of PK assessment, or during the month 1, 2 or 3 for FIX activity if feasible.
I: If subjects receive treatment for less than 3 doses per month, they may omit the site visit(s) until they received ~5 doses.
J: FIX activity tested at local Laboratory if no previous FIX data available in medical records.
K: Subject eDiary may be dispensed once caregiver is trained to administer rIX-FP at home, but not prior to receiving a minimum of 10 infusions at the study center or medical facility under medical supervision.
L: End of Study for Arm 4 is the Month 36 visit.

3.6 Study Populations

The following analysis populations will be used for the presentation of the statistical analyses in this study.

3.6.1 Screened Population

The screened population will consist of all subjects who provided written informed consent to undergo study screening procedures.

3.6.2 Enrolled Population

The enrolled population will consist of all subjects who were not screen failures.

3.6.3 Safety Population

The safety population will consist of all subjects who received at least 1 dose of rIX-FP in this extension study.

3.6.4 Efficacy Population

The efficacy population will consist of all subjects in the safety population. Data collected in the surgery and subcutaneous substudies will be excluded from analyses of efficacy. Analyses of the surgery and subcutaneous substudies will be reported separately.

3.6.5 Per-protocol Population

The per-protocol (PP) population will include all subjects in the efficacy population who complete the study without any major protocol deviations that would affect the assessment of the efficacy endpoints. In some cases, subjects may be retained in the PP population with partial data up to the point of a major deviation.

If a subject did not treat a bleeding event per the protocol, the PP analysis may exclude the bleeding event in question, rather than all of the subject's data. Accordingly, the following events will be assessed for exclusion from the PP analysis:

- use of a FIX product other than rIX-FP to treat a bleeding event;
- treating a bleeding event ≥4 hours after the start of the bleeding event, or
- treating a bleeding event that occurred more than x days and 12 hours after the subject's last prophylaxis dose in the x-day schedule, where x is either 7, 10 or 14

A full review of the bleeding event data will be performed to identify other bleeding events that should be removed from the analysis. Rationale for the reason these bleeding events are being excluded will be provided in the data review meeting minutes before database lock.

No PP population will be used for analysis of PUP data.

3.6.6 Pharmacokinetic Population

The PK population will include subjects who have received at least 1 dose of rIX-FP for the purpose of PK sampling and have a sufficient number of analyzable PK blood samples (i.e. at least 1 PK parameter calculated) for PK assessment of rIX-FP. Subjects will be excluded from the analysis if an insufficient number of analyzable PK samples were obtained to permit the evaluation of at least 1 PK parameter for rIX-FP, excluding samples obtained after receiving a dose of rIX-FP or any other FIX product for the treatment of a bleeding event during the PK sampling period.

3.6.7 Surgical Population

The surgical population, which is a subset of the safety population, will include all PTPs who have provided written informed consent for the surgery substudy and received at least 1 dose of rIX-FP for any surgical procedure in this substudy.

3.6.8 Subcutaneous Safety Population

The subcutaneous safety population, which is a subset of the safety population, will include all PTPs who have provided written informed consent for the SC substudy and received at least 1 SC dose of rIX-FP in the SC substudy.

3.6.9 Subcutaneous PK Population

The subcutaneous PK population will include all PTPs who received at least 1 SC dose of rIX-FP and have blood samples drawn for PK assessment. Subjects will be excluded from analysis if an insufficient number of analyzable PK samples were obtained to permit the evaluation of at least 1 PK parameter for rIX-FP, excluding samples obtained after receiving an IV dose of rIX-FP or any other FIX product for the treatment of a bleeding event during the PK sampling period.

3.7 Randomization

This is a non-randomized study.

3.8 Blinding

This is an open-label study.

4 Statistical Analyses and Methods

4.1 General Considerations

Data management and statistical analyses will be performed by a contract research organization (CRO) under the supervision of CSL Behring. Data manipulations and statistical analyses will be performed using SAS® version 9.2 (SAS Institute) or higher.

Continuous variables will be summarized in terms of the mean, standard deviation (SD), median, minimum and maximum. Other descriptive statistics (e.g., quartiles, coefficient of variation) may be reported when appropriate. Categorical variables will be summarized using frequency counts and percentages. In general, data will be summarized by treatment arm, regimen, regimen within treatment arm (as applicable), age group, and will include the total subjects in the population. Unless otherwise noted, the denominator for the percentages will include all subjects in the respective treatment arm or regimen. Tables, listings and figures will be presented separately for PTPs and PUPs unless otherwise noted. Analyses of data for PUPs will be descriptive unless otherwise stated.

Unless otherwise specified, all confidence intervals (CIs) and p-values will be two-sided.

For summary tables of PTP data by regimen, the 10-day regimen will be combined with the 3x-per-month regimen. The only exception will be for disposition where separate counts and percentages will be displayed for these regimen groups as collected on the eCRF.

Supportive listings for summary tables and figures will be provided as applicable.

4.1.1 Summaries by Age Categories

In general, summaries by age for PTPs will include age <12 years and ≥12 years. In some cases, e.g. for 4 months safety update, additional age categories (age <6 years, ≥ 6 years to <12 years, ≥12 years to <18 years, <18 years and ≥18 years) will be needed.

Summaries by age for PUPs will include age <6 years and ≥6 years to ≤12 years.

4.1.2 Reference Date and Study Day

Timing of all safety events, interventions, and findings will be relative to the reference date. The reference date for this study will be the date of the first dose of study medication in this study.

Study day for events on or after the date of the first dose will be defined as the number of days from the date of the first dose of study medication in this study, plus 1 day. Therefore, the date of the first dose will be defined as Day 1. For events before the date of the first dose, study day will be derived as the difference in days between the date of the first dose and the date of interest. Thus, the day before the date of the first dose will be defined as Day –1.

4.1.3 Baseline and Changes from Baseline

Unless otherwise indicated, baseline is defined as the Day-1 visit in this study, and baseline assessments are the last assessments prior to the first dose of study medication in this study. Assessments that are obtained after the first dose of study medication in this study will be considered post-baseline values. If measurement of a variable is not made on a given subject prior to the first dose of study medication, then that subject will be considered not to have a baseline value for that variable. Change from baseline is defined as post-baseline assessment minus baseline assessment.

4.1.4 Handling Partial Dates

A date is a valid partial date if the day is missing, or the day and the month are missing. Where appropriate, the algorithm outlined in Table 1 will be applied to accommodate partial dates.

Table 1. Handling of Partial Dates

Date	Missing Element(s)	Imputation
Start date	Day and Month	First day of the year
	Day	First day of the month
Stop date	Day and Month	Last day of the year
	Day	Last day of the month

4.1.5 Early Withdrawal or Missing data

Apart from the handling of partial dates, and derivation of annualized bleeding rates, no imputation due to withdrawals or missing data will be applied for analyses of efficacy or safety endpoints.

4.1.6 Adjustment for Covariates

There will be no adjustments for covariates.

4.1.7 Considerations for Multi-Center Studies

There will be no pooling of centers for the purpose of analyses.

4.2 Study Periods

This section outlines the separate study periods that are defined for the purposes of the statistical analyses. The study periods are based on the reason for dosing of CSL654 in this study. The PK, prophylaxis treatment, surgery substudy and subcutaneous substudy periods will be treated as disjoint periods for the analyses of efficacy.

The partitioning of the PK, prophylaxis treatment, and substudy periods is described in Table 2.

Table 2. Study periods

Period	Definition
PK	*Start*: administration of the first rIX-FP infusion in the PK evaluation period. *Stop*: at the time of the last PK sample; or when the subject received a FIX product for prophylaxis; whichever occurred first.
x[a] day prophylaxis regimen	*Start*: administration of the first *x*-day infusion. *Stop*: *x* days after the last rIX-FP infusion, or with the administration of a different regimen, or start of another period, or at the End of Study visit; whichever occurred first.
Surgery Substudy	Surgery (Major or Minor) *Start*: administration of first rIX-FP infusion in preparation for the surgery. *Stop*: 28 days after the surgical procedure or at the End of Substudy visit for the surgery substudy, whichever occurred last.

[a] x denotes 7, 10, 14, or 21 (-day prophylaxis regimen).

4.3 Interim Analyses

No formal interim analyses are planned for the purpose of futility or safety stopping.

The following analyses are planned before study closure:

1. The final analysis of the SC substudy data will be conducted when all subjects have completed the SC substudy.
2. The final analysis of PTPs (including surgical data from PTPs) will be conducted when all PTPs have completed the study.

In addition, safety, efficacy and/or PK data and subject characteristics may be reported to regulatory authorities before study closure.

4.4 Disposition of Subjects

Subject disposition (subject populations) will be presented for all subjects who participated in the study by study arm, age (as described in Section 4.1.1) and total. Summaries of disposition will include the number of enrolled subjects and counts and percentages of subjects in the safety, efficacy, PP, PK, surgical, and SC safety populations, if applicable. Additionally, the summary of disposition will include counts and percentages of subjects' initial and final assigned prophylaxis regimens overall and by regimen (3-day, 7-day, 10-day, 14-day, and 21-day, if applicable).

The number and percentage of subjects who completed the study and did not complete the study, and the reasons for discontinuation from the study (adverse event(s), death, lack of efficacy, lost to follow-up, other, physician decision, protocol violation, study terminated by sponsor, and withdrawal by subject) will be summarized. Reasons for study discontinuation for PUPs due to adverse event, protocol violation, and other (specified) will be further categorized as those associated with Coronavirus Disease 2019 (COVID-19) and those not associated with COVID-19. Other reason specified will be based on free text containing the terms "COVID" or "Coronavirus".

The total number of screened subjects and screen failures also will be displayed. Subject disposition will be summarized separately for all PTPs and all PUPs.

A separate summary of subjects who failed screening including the total number of subjects screened for the study, the count and percentage of subjects who failed screening, and the reasons for failing screening (screen failure, adverse event, lost to follow-up, physician decision, protocol violation, and withdrawal by subject) will be provided.

Summaries of subject populations by geographic region and country of enrollment will be provided by arm, age group, and total. Country will be displayed within region (North American, Europe, Middle East, Asian Pacific, Oceania, and Africa, if applicable). For each country, the number and percentage of subjects enrolled, and the number and percentage of subjects in each of the safety, efficacy, PP, PK, surgical, SC safety, and SC PK populations will be displayed, if applicable.

Deviations from the inclusion/exclusion criteria will be summarized for all enrolled subjects. The summary will include counts and percentages of subjects with any deviation from inclusion criteria and counts and percentages of subjects with deviations from each inclusion criteria as applicable. A similar display will be provided for the exclusion criteria.

4.5 Protocol Deviations

The number and percentage of subjects who had major protocol deviations will be summarized for the safety populations.

Major protocol deviations will be summarized by categories of deviation. All protocol deviations that have been documented throughout the study will be presented in subject listings for all subjects. Separate listings will be provided for PTP subjects in the efficacy population who were excluded from the PP population.

The summary of major protocol deviations for PUPs will also include deviations associated with COVID-19, and the source listing will flag these deviations.

Planned visits that were missed due to COVID-19 will be summarized for PUPs. A by-subject listing of missed visits due to COVID-19 will be included.

4.6 Demographics and Baseline Characteristics

Demographics will be summarized using frequency counts and percentages for the following: race (American Indian/Alaska Native, Asian, Black or African American, Native Hawaiian/Other Pacific Islander, White, and Other), ethnicity (Hispanic or Latino, not Hispanic or Latino, not reported, and unknown), body mass index (BMI) category (<30 kg/m^2 and ≥ 30 kg/m^2), and Geographic Region. Age, height, weight, and (BMI at screening for this study will be summarized with descriptive statistics for continuous variables.

Age will be determined for all subjects using date of birth and the date of informed consent (i.e. date of screening) for Study CSL654_3003. The value of age will be derived as the integer part of (date of screening – date of birth)/365.25. Partial dates will be handled as outlined in Table 1. BMI will be derived as weight (kg) / (height (m))^2. BMI will be summarized as a continuous variable and categorized as <30 kg/m^2, ≥ 30 kg/m^2.

Demographics will be summarized for the Safety population overall, and presented by arm, age group (as described in Section 4.1.1), and total.

The following baseline characteristics will be summarized and/or listed for PTPs and PUPs:

- Medical/surgical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 21.0 for PTPs and Version 23.1 or higher for PUPs. These will be presented by system organ class (SOC) and preferred term (PT). Past as well as active (i.e., still on-going at Screening) medical histories will be displayed.
- Hemophilia B history as follows:
  - time (months), defined as (date of informed consent – date of diagnosis)/30.4375, since first diagnosis of severe hemophilia B (factor IX activity ≤2%) (for Arm 3) or time (months) since first diagnosis of moderate to severe hemophilia B (for Arm 4)
  - time (weeks) since last factor IX level < 2% - Arm 3 only
  - estimated or documented treatment days (EDs) at the screening visit – Arm 3 only
  - number and type of bleeding events in the past 12 months – Arm 3 only
  - family history of inhibitors against FIX – Arm 4 only
  - genotype
  - presence of chronic hemarthrosis, hemophilic arthropathy, and target joints

The following baseline characteristics will be summarized and listed only for subjects in Arm 3:

- FIX treatment history including
  - time since receiving the first prophylaxis treatment (months)
  - most recent treatment modality (prophylaxis, on-demand) prior to study entry
  - modalities (routine prophylaxis, on-demand, post-surgery, and prevention prior to activity) in the previous 6 months
  - product type (pdFIX, rFIX, or rIX-FP) in the previous 6 months
  - IU/kg per prophylaxis dose and times per week for prophylaxis in the previous 6 months
  - weekly prophylaxis dose (IU/kg) (calculated as IU/kg per prophylaxis dose multiplied by times per week) in the previous 6 months
  - IU/kg for on-demand in the previous 6 months; and
  - total dose [IU] administered in the previous 6 months

4.7 Exposure Days and Time on Study

The number of EDs will be calculated in the clinical database for each subject and reported for both this study and over the cumulative experience of rIX-FP exposure in lead-in studies (CSL654_3001 and CSL654_3002). Exposure to rIX-FP will be summarized separately for the substudies (surgery and SC). An ED of rIX-FP is defined as any day that the subject receives an infusion of rIX-FP regardless of the number of infusions on that day or reason for the infusion(s). The number of EDs for subjects in lead-in studies will be imported into the CSL654_3003 database and used to summarize the total number of EDs across studies.

Summaries of exposure and time on study will include descriptive statistics for time on study in days, weeks, and months, total EDs in Study CSL654_3003, total EDs across all CSL654 studies, and the total dose (IU, IU/kg, IU/kg/year, and IU/kg/month) of CSL654 received only in Study CSL654_3003, and will be displayed by arm, prophylaxis regimen, age groups (Section 4.1.1), and total.

The number of EDs will be summarized as both a continuous and categorical variable. The following categories will be presented as applicable for PTPs:

- < 50 EDs
- ≥ 50 EDs
- ≥ 75 EDs
- ≥ 100 EDs
- ≥ 150 EDs
- ≥ 200 EDs

- ≥ 250 EDs
- ≥ 300 EDs

The following categories will be presented as applicable for PUPs:

- < 50 EDs
- ≥ 50 EDs
- ≥ 75 EDs
- ≥ 100 EDs

For PTPs, cumulative distribution function (CDF) plots will be provided for EDs in CSL654_3003 and total EDs across all CSL654 lead-in studies. The CDF plots will be generated using the PTP safety population.

For PUPs, CDF plots will be provided for EDs in CSL654_3003 PUPs. The CDF plots will be generated using the PUP safety population.

Time on study

Time on study (days) will be derived as:

(end of study visit date) – (study medication start date) + 1.

Time on study in months will be calculated as:

Time on study (days) / 30.4375

Exposure and time on each regimen will be the sum of accumulated time (in days) on each regimen in Study CSL654_3003. For example, a subject who was on the 7-day regimen for 20 days, followed by 30 days on the 14-day regimen, and then went back on the 7-Day regimen for 20 days will have a total of 40 days on the 7-day regimen.

Descriptive statistics will be provided for exposure and time on study by prophylaxis regimen and age for the PTP safety population overall and for the following subgroups:

- subjects ≥18 years in the PTP safety population who switched from 14-day regimen to 21-day regimen
- subjects in the PTP safety population who switched to the 21-day regimen (subjects ≥18 years are allowed to switch to 21-day regimen) compared with subjects ≥12 years in the PTP safety population who did not switch to the 21-day regimen
- subjects <12 years in PTP safety population on 14-day regimen
  - paired comparisons for subjects who participated in both the 7-day regimen and 14-day regimen in 3003 (total 14-day regimen in 3003 presented on the same display)
  - paired comparisons for subjects who participated in the 7-day regimen in 3002 and/or 3003 and the 14-day regimen in 3003

- subjects <12 years in the PTP safety population who switched to the 14-day regimen compared with subjects <12 years in the PTP safety population who did not switch to the 14-day regimen

Descriptive statistics will be provided for time on study, exposure days, and total dose (IU and IU/kg) by age group (<6 vs 6 to ≤12 years) for the PUP safety population.

Subject listings of exposure for PTPs will include the number of EDs, duration on study (days, weeks, and months), and total dose (IU, IU/kg, IU/kg/year, and IU/kg/month) in Study CSL654_3003. Listings of rIX-FP administration for PTPs will include location of administration (home, hospital, or study site), infusion start date and time, lot, lot number, and vial, total IU administered, by whom treatment was administered (subject, caregiver, or site staff), calculated dose administered (IU/kg), reason for administration, and new bleeding event.

Subject listings of exposure for PUPs will include total dose (IU), number of EDs, and duration on study (days, weeks, and months). Listing of rIX-FP administration for PUPs will include location of administration (home, hospital, or study site), infusion start date and time, log, lot number, vial number, total dose (IU) administered, treatment operator (subject, caregiver, or site staff), calculated dose administered (IU/kg), and reason for administration, If it's for a bleeding event, whether it's for a new bleeding event or an existing bleeding event will be indicated.

Listings of rIX-FP administration will be provided separately for the surgery and SC substudies for the PTP safety population

4.8 Dose Assignment and Adjustment

For PTPs, dose assignment by age (as described in Section 4.1.1) and overall will be presented by prophylaxis regimen and will include summary statistics for the initial (first) assigned dose (IU/kg), most recent assigned dose (IU/kg), and all assigned doses (IU/kg).

Prophylaxis regimen shifts for PTPs will be presented by regimen and age. Displays will include the initial and final regimen for each study arm. PTPs who participated in multiple regimens will be counted only once in each regimen in which they participated. Prophylaxis regimen shifts will not be summarized for PUPs.

For PUPs, dose assignment by age and overall will be presented for the following items:

- Initial and most recent assigned prophylaxis dose (IU/kg)
- All assigned prophylaxis doses (IU/kg)
- Initial and most recent assigned on-demand dose (IU/kg)
- All assigned on-demand doses (IU/kg)
- Initial and most recent dose received (IU/kg)
- All doses received (IU/kg)

Subject listings for dose assignment and adjustment will include assigned dose, prophylaxis regimen, on-demand dose assignments for joint/muscle and/or deep muscle as applicable, dose and frequency adjustments, modality, and reason for dose adjustment.

4.9 Prior and Concomitant Medications and Nonpharmacological Interventions

Prior medications are medications that started and stopped before the first dose of study medication. Concomitant medications are all medications taken during the study, including medications started before and ongoing at first dose of study medication. All medications will be considered concomitant for subjects in Arms 1 and 2 of the study.

The analysis datasets associated with concomitant medications will contain the flags defined below:

Prior: the start date is either strictly prior to the reference date or missing.

Concomitant: either the start date or the end date is on or after the reference date, or the end date is missing.

Partial dates will be handled as outlined in Table 1. Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug dictionary (March 2018, Enhanced B for PTPs and September 2020, Enhanced B or higher for PUPs). Summaries of prior and concomitant medications will include the preferred drug name within the Anatomical-Therapeutic-Chemical (ATC) level 4 classification and will be presented by arm, age group (Section 4.1.1), and total. If a subject has multiple occurrences of a medication, then the subject will be presented only once in the respective subject count.

A separate summary of concomitant medications will be provided for PUPs who administer medications for treatment of an adverse event associated with COVID-19. These medications will be flagged in the source listing for concomitant medications.

Subject listings will be provided for nonpharmacological interventions (e.g., physical therapy or a minor surgical procedure performed outside of the surgery substudy) as collected on the eCRF.

4.10 Compliance

Compliance will be defined in terms of the following:

- the prophylaxis schedule, i.e., “prophylaxis compliance”
- the prescribed dose, i.e., “dose compliance”
- time to on-demand treatment of a bleeding event, i.e., “treatment compliance”

Prophylaxis compliance will be derived as:

(number of on-schedule prophylaxis infusions received) /
(expected number of prophylaxis infusions) * 100

where on-schedule prophylaxis infusions are injections during the study period that occur at the expected dosing frequency within the defined target days.

Dosing for each prophylaxis regimen is expected to occur within the following target days:

- For the 7-day regimen, injection of rIX-FP occurs between days 6 and 8 after the previous prophylaxis injection.
- For the 10-day regimen (i.e., every 10 days or 3 times per month), injection of rIX-FP occurs between days 8 and 11 after the previous prophylaxis injection.
- For the 14-day regimen, injection of rIX-FP occurs between days 13 and 15 after the previous prophylaxis injection.
- For the 21-day regimen, injection of rIX-FP occurs between days 20 and 22 after the previous prophylaxis injection.

Dose compliance will be derived as follows:

(number of doses within 10% of the prescribed dose) / (number of doses received) * 100

Treatment compliance will be derived as follows:

(number of bleeding events treated with rIX-FP <4 hours after the start of a bleeding event) / (number of bleeding events treated with rIX-FP) * 100

A subject will be defined as compliant with each of prophylaxis regimen, dose, or treatment if the compliance is ≥80%.

Prophylaxis, dose, and treatment compliance will be summarized by prophylaxis regimen in the safety population using descriptive statistics. Summaries will include the number and percentage of subjects who were compliant with their prophylaxis regimen, dose, treatment, the number and percentage of subjects who were ≥80% for each measure, and the number and percentage of subjects who were <80% compliant for each measure. Subject listings that include all derived compliance measures for each subject will be provided.

4.11 Consumption of rIX-FP

Consumption of rIX-FP during routine prophylaxis will be summarized with descriptive statistics by regimen, age group (Section 4.1.1), and geographic region.

Summaries of consumption of rIX-FP will include the following measures:

- number of prophylaxis injections per subject (per month and per year)
    - per year calculated as: sum of prophylaxis injections per subject*365.25/(total duration)
    - per month calculated as: sum of prophylaxis injections per subject*(365.25/12)/(total duration)
- monthly prophylaxis dose (IU/kg) calculated as: sum of prophylaxis dose (IU/kg) per subject*(365.25/12)/(total duration)

- weekly prophylaxis dose (IU/kg) calculated as: IU/kg per prophylaxis dose x times per week of the most recent treatment modality

Standardized yearly totals will be calculated for each subject by calculating the following:

subject's observed total *365.25/(total duration).

Standardized monthly totals will be calculated by dividing the yearly estimate by 12.

Calculation of duration will be based on the periods defined in Table 2.

Consequently, periods when the subject is not receiving routine prophylaxis or treatment for any bleeding events including preventative injections (e.g. PK) will not be included in summaries of prophylaxis consumption.

Each dose (IU/kg) will be calculated using the total international units (IU) of rIX-FP recorded and the most recent recorded value of weight (kg).

4.12 Efficacy Analyses

4.12.1 Bleeding Events

A bleeding event starts with the first sign of a bleeding event, and ends 72 hours after the last treatment for the bleeding event. Any symptoms of bleeding at the same location and of the same type, or for which infusions are administered less than 72 hours apart, are considered the same bleeding event. A "bleeding event" dose taken more than 72 hours after the preceding dose will be considered the first infusion to treat a new bleeding event at the same location, and the new bleeding event will be classified as type = "unknown". A bleeding event at a different location will be considered a separate bleeding event regardless of the time from the last infusion.

A "bleeding event" dose indicated as being taken within 30 minutes prior to the bleeding event will be counted towards that bleeding event with time to treatment set to 0 minutes. Multiple infusions administered within 3 hours of one another on the same calendar day will be counted as a single infusion with total combined IUs.

Infusions needed to achieve hemostasis will count towards the treatment of a bleeding event. Preventative and additional doses will not count toward the treatment of a bleeding event.

- A preventative dose is defined as a dose taken prior to physical activity or physical therapy to prevent or minimize a bleeding event.
- An additional dose is defined as a dose that is taken beyond the need to control hemostasis.

Prophylaxis doses will count towards the treatment of a bleeding event only under the following conditions:

- if given within 24 hours after the start time of the last on-demand dose for a "bleeding event"

- if treated with rIX-FP and dose for "bleeding event" is not indicated and a "prophylaxis" dose of rIX-FP is administered within 24 hours

Bleeding events during the PK period will count towards the treatment of a bleeding event if the bleeding event was treated with rIX-FP, but they will otherwise be excluded from analysis.

Bleeding events during the surgical, subcutaneous, and PK period will be excluded from analysis.

4.12.2 Primary Efficacy Analysis

This study does not have a primary efficacy endpoint since the evaluation of efficacy is not a primary objective.

4.12.3 Secondary Efficacy Analyses

ABRs will be derived for total, spontaneous, joint, traumatic, and unknown bleeding event types for each subject by prophylaxis regimen. AsBR and ABR are secondary efficacy endpoints in this study. CCI [redacted]. In general, ABRs will be derived for each subject as follows:

(number of bleeding events) / (observed treatment period of interest days) * 365.25.

Only bleeding events requiring treatment with CSL654 will be included in the derivation of bleeding rates. The ABR will be estimated for subjects who complete at least 12 weeks of treatment on the given regimen using the subject's observed data. If the subject does not complete at least 12 weeks of treatment on the given regimen or has no treated bleeding events while on the given regimen, then the ABR will be considered missing.

The following periods of time (Table 2) will be excluded from derivation of bleeding rates for prophylaxis regimens:

- during the surgery substudy period
- during the SC substudy period
- during the PK period

Analysis of the surgery and SC substudies are not applicable to PUPs. ABRs will be derived for subjects in Arm 4 and will be listed by category per subject and regimen.

Disjoint periods of observation with the same prophylaxis regimen will be combined within a subject. Duration of treatment for each period will begin when the subject receives the first dose of the assigned prophylaxis regimen. ABRs will be summarized across PTP subjects using descriptive statistics.

Calculation of duration for bleeding rates will be based on the treatment periods defined in Table 2. Consequently, periods when the subject is not receiving treatment for any bleeding event or prophylaxis treatments, will not be included in the summaries.

ABRs will be summarized across subjects using descriptive statistics. A crude rate will be calculated for each regimen and also for the ratio of regimens along with an exact two-sided 95% CI under the assumption that counts follow a negative binomial distribution (Zeger SL and Liang KY, 1986). The analyses will be repeated for sensitivity assuming a Poisson distribution. The p-values from these models will not be presented.

A comparison of AsBR and ABR will be performed between 14-day prophylaxis treatment in this study compared with on-demand treatment from Study CSL654_3001 (Arm 2) for subjects ≥12 years of age. A Wilcoxon signed-rank test will be performed on the matched-pairs at the two-sided 0.05 level to test the hypothesis that the median difference in rates differs from zero. Descriptive statistics will also be provided.

The analysis of AsBR will also be conducted with subjects from both Study CSL654_3001 and the present extension study to assess whether a similar treatment effect exists between the 7-day prophylaxis regimen and the 14-day prophylaxis regimen.

In order to demonstrate that a similar treatment effect also exists for the 14-day prophylaxis regimen and 7-day prophylaxis regimen, the mean AsBR will be compared between the two prophylaxes regimens to evaluate non-inferiority. Following the same clinically acceptable difference used in the CSL654_3001 study, a non-inferiority margin of 6 spontaneous bleeding events/year is used.

The null and alternative hypotheses for the AsBR are as follows:

H_0: $\mu_{7\text{-day}} - \mu_{14\text{-day}} \leq -6$

H_1: $\mu_{7\text{-day}} - \mu_{14\text{-day}} > -6$

To establish non-inferiority of the 14-day regimen versus the 7-day regimen for the AsBR, the lower confidence limit of the 95% CI, based on a paired t-test, for the difference between the two means (7-day regimen – 14-day regimen) must be greater than -6 spontaneous bleeding events/year. In addition, a 99% CI of the mean difference will be assessed.

The same analyses will be conducted for the comparison of ABR. Furthermore, the non-inferiority analysis of AsBR and ABR between the 14-day or 10-day regimens (combined) and the 7-day regimen will be conducted in a similar manner.

Summaries of ABRs by regimen will be presented from Study CSL654_3003. In addition, when comparing the ABRs between regimens, the bleeding data from the lead-in studies (CSL654_3001 and 3002) and this study will be utilized as applicable.

Additionally, the number and percentage of subjects with no bleeding events, number and percentage of subjects with at least 1 bleeding event (requiring treatment with CSL654 and not requiring treatment with CSL654) will be presented.

Analyses of bleeding rates will be presented overall, by age, and by regimen as applicable. Hypothesis testing will not be applicable for PUPs.

A summary of the secondary statistical analyses that will be performed for annualized bleeding rates is provided in Table 3.

Table 3. Secondary Analyses of Annualized Bleeding Rates

Population	Statistic	Hypothesis	Endpoints	Analysis
PTP Efficacy population	Descriptive for regimen Point estimate and 95% CI based on negative binomial and Poisson regression***	N/A	ABR AsBR	prophylaxis regimens [7, 10, 14, and 21 days]**
Primary Efficacy Population from 3001*	Descriptive for regimen and intra-subject ratio p-value from Wilcoxon signed rank based on intra-subject ratios Point estimate & 95% CI for regimen based on negative binomial and Poisson regression***	H_0: $\mu_{14\text{-day}} / \mu_{\text{on-demand}} \geq ½$ versus H_1: $\mu_{14\text{-day}} / \mu_{\text{on-demand}} < ½$ N/A	ABR AsBR	14-day regimen in 3003 Arm 2 vs On-demand regimen in 3001
Subjects ≥12 years in PTP Efficacy Population	Point estimates & 95% CIs Based on paired t-test Point estimate & 95% CI for regimen based on negative binomial and Poisson regression***	Non-inferiority: H_0: $\mu_{7\text{-day}} - \mu_{14\text{-day}} \leq -6$ H_1: $\mu_{7\text{-day}} - \mu_{14\text{-day}} > -6$ H_0: $\mu_{7\text{-day}} - \mu_{10 \text{ or } 14\text{-day}} \leq -6$ H_1: $\mu_{7\text{-day}} - \mu_{10 \text{ or } 14\text{-day}} > -6$ N/A	ABR AsBR	14-day regimen in 3001* and 3003 vs 7-day regimen in 3001* and 3003 and 14- or 10-day regimen in 3001* and 3003 vs 7-day regimen in 3001* and 3003

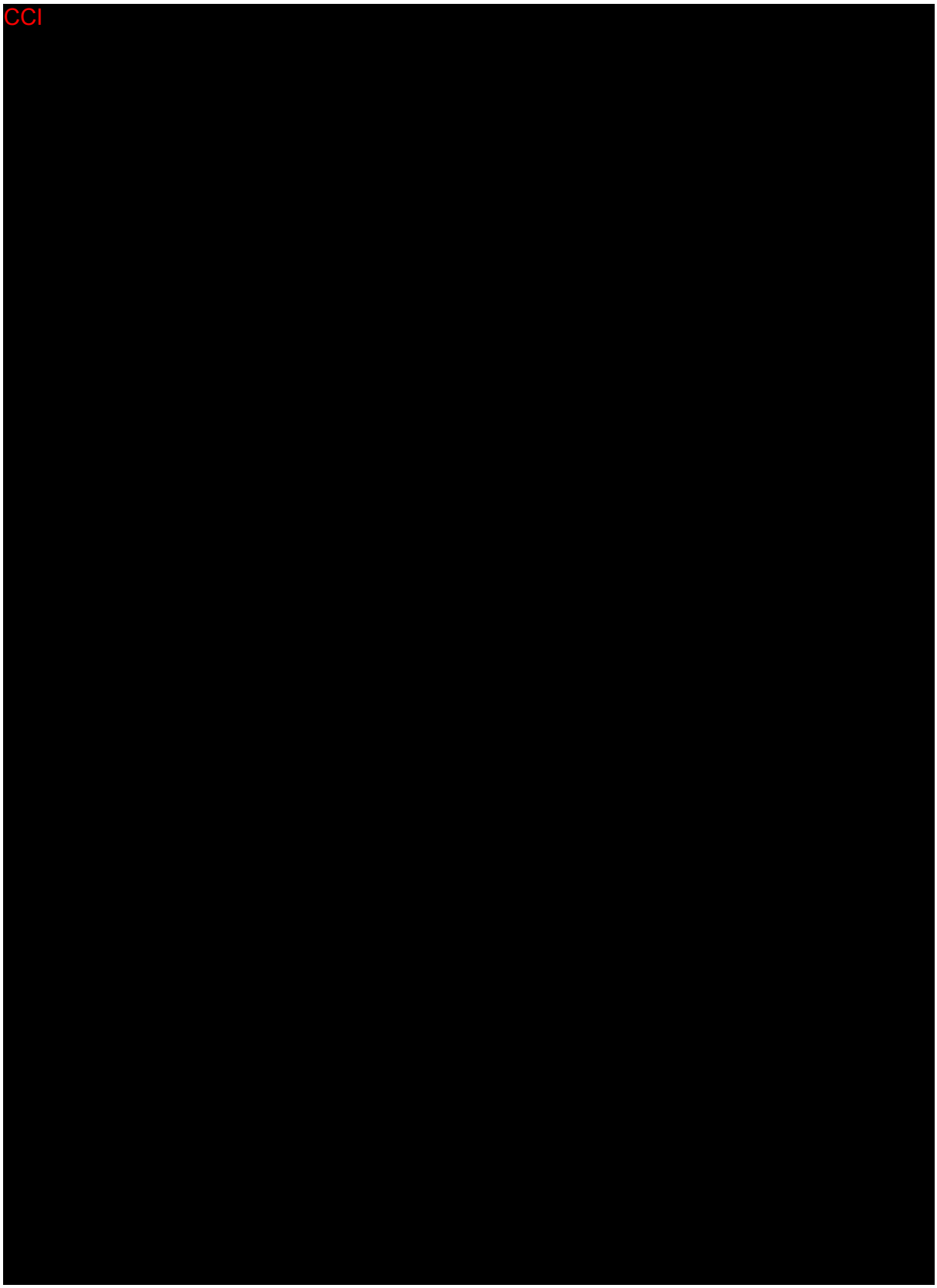
Note: ABR = Annualized Total Bleeding Rate, AsBR = Annualized Spontaneous Bleeding Rate;
*On-demand treatment data from Study CSL654_3001 available for subjects in CSL654_3003
**See Table 2

***Generalized linear modeling using SAS' GENMOD procedure will be utilized (Zeger SL and Liang). The Poisson distribution with log link function or negative binomial distribution, and log_time offset will be specified. The p-value from this analysis will not be presented.

Multiple comparisons and multiplicity

A fixed-sequence, multiple testing procedure will be used to control the overall type I error rate at the two-sided 0.05 level for the analyses of ABRs detailed in Table 3. Specifically, the test of non-inferiority between the 14-day prophylaxis regimen and on-demand will first be performed at the two-sided 0.05 level of significance. Only if the 14-day regimen is found to be effective (i.e. the alternative hypothesis H_1 is concluded), will the test of non-inferiority of the 14-day prophylaxis regimen to the 7-day prophylaxis regimen at the pre-specified margin be performed. Only if the 14-day prophylaxis regimen is demonstrated to be non-inferior to the 7-day regimen, will the non-inferiority test of the extended prophylaxis regimen (10-day or 14-day) with the 7-day regimen be performed.

CCI



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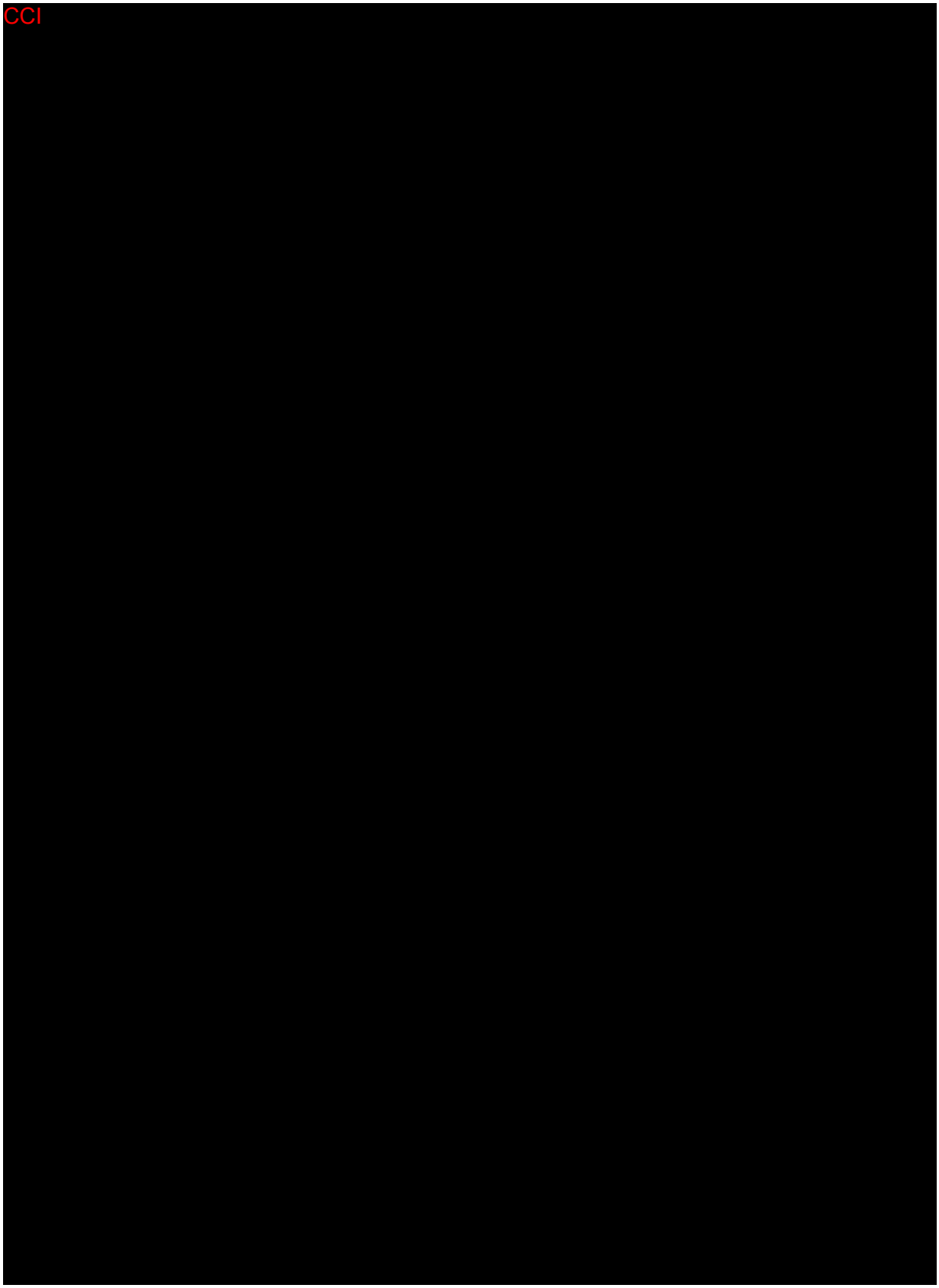

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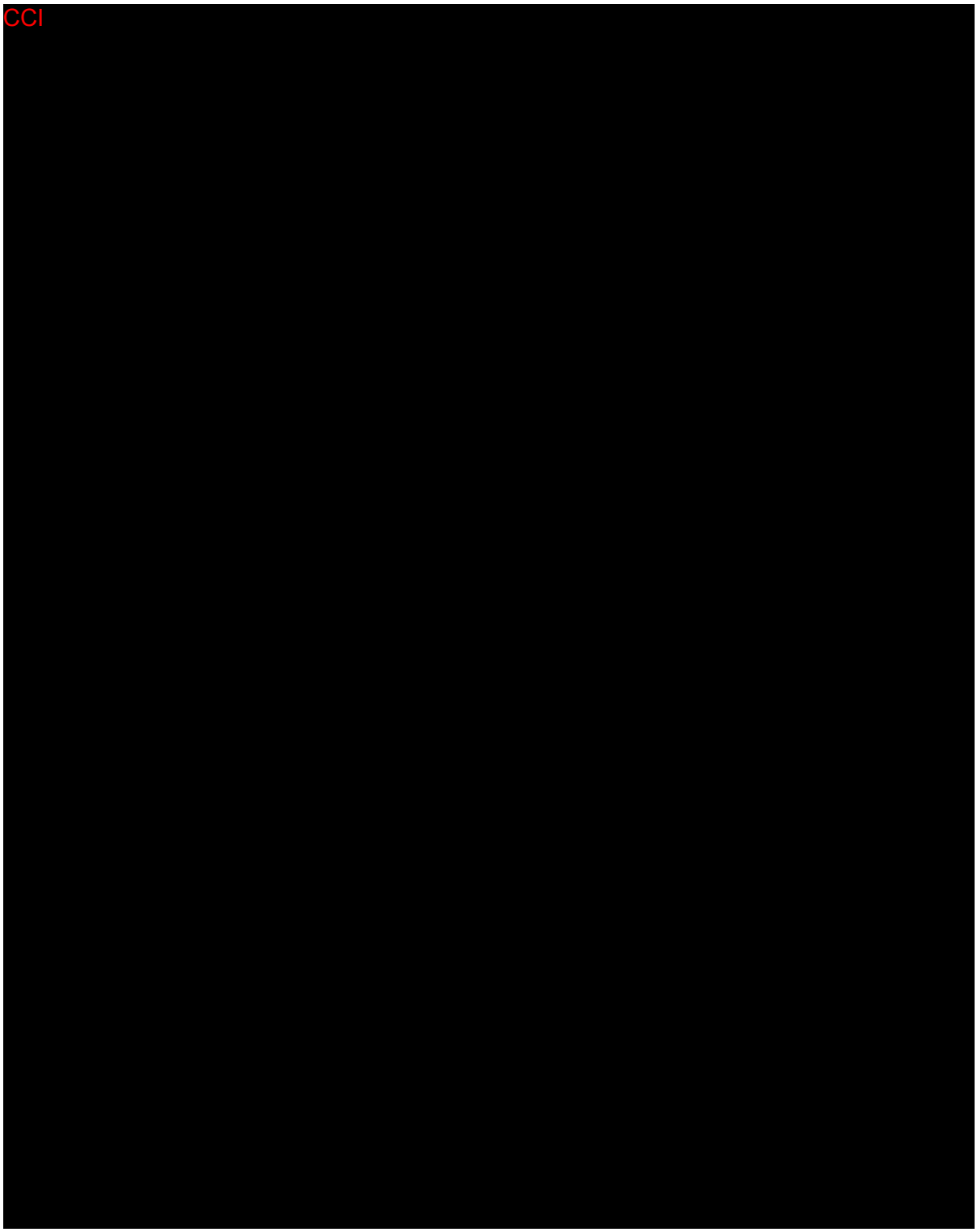

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[redacted]

4.12.5 Efficacy in the Surgery Substudy

Details of the analysis of efficacy during the surgery substudy are presented in Section 6.

4.13 Safety Analyses

Safety summaries will be performed on the safety population unless otherwise specified. No formal statistical comparisons for safety will be made unless specified.

Safety will be assessed on the basis of the following:

- inhibitors against FIX
- adverse events (AEs)
- local tolerability
- laboratory safety parameters (serum chemistry and hematology)
- antibodies against rIX-FP and Chinese hamster ovary (CHO) cells

- vital signs
- physical examination

4.13.1 Primary Safety Analysis

The primary safety endpoint is the development of inhibitors against FIX.

4.13.2 Inhibitors to Factor IX

The development of inhibitors against FIX is defined as any inhibitor identified at a concentration of ≥0.6 BU/mL and confirmed with retesting to be ≥0.6 BU/mL. Both tests must be performed by a central laboratory. The incidence of inhibitor development and 95% CI will be presented separately for PTPs and PUPs in the safety population.

For estimating the incidence of inhibitors against FIX in PTPs, the numerator will include all PTPs with inhibitors regardless of EDs to rIX-FP; the denominator will include PTPs with at least 100 EDs plus PTPs with less than 100 EDs who have confirmed inhibitors as follows:

> Incidence of inhibitors against FIX in PTPs = number of PTPs who develop inhibitors up to 100 EDs (i.e. ≤100 EDs) / (total number of PTPs with at least 100 EDs (≥100 EDs) + PTPs with <100 EDs who have confirmed inhibitors)
>
> PTPs not tested at ≤100 EDs will be excluded.

For estimating the incidence inhibitors against FIX in PUPs, the numerator will include all PUPs with inhibitors to rIX-FP; the denominator will include PUPs with at least 50 EDs plus PUPs with less than 50 EDs but with confirmed inhibitors as follows:

> Incidence of inhibitors against FIX in PUPs = number of PUPs who develop inhibitors / (PUPs with ≥50 EDs + PUPs with <50 EDs who have confirmed inhibitors).
>
> PUPs not tested at ≤50 EDs will be excluded.

The number of subjects with antibodies to plasma-derived factor IX (pdFIX), recombinant FIX (rFIX), albumin, and CHO cells will be tabulated by age group and total.

The two-sided Wald and Clopper-Pearson 95% CI (Clopper C, Pearson ES) for the incidence of FIX inhibitor formation will be provided. Example SAS code for this procedure is provided below:

```
proc freq data=data order=freq;
   tables inhibitor / binomial(exact);
   weight pos;

run;
```

4.13.3 Adverse Events

As per the International Council for Harmonisation (ICH) guidelines, an AE is any untoward medical occurrence in a subject 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. Bleeding events will not to be considered as AEs but are to be documented as a bleeding event in the subject's electronic diary (eDiary).

Any AE reported as resulting in death, immediate risk of death (life threatening), inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, or a congenital/anomaly/birth defect will be classified as a serious adverse event (SAE). An SAE may also be any other medically important event that, in the opinion of the Investigator, may jeopardize the subject or may require intervention to prevent 1 of the other outcomes listed above.

Adverse events (AEs) will be coded and grouped by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) Version 21.0 for PTPs and Version 23.1 or higher for PUPs.

An AE will be regarded as treatment emergent (TEAE) for this study if it was present before the first dose of rIX-FP in this study and subsequently worsened in severity, or was not present before the first dose but subsequently appeared. For this determination, partially missing dates will be handled as outlined in Table 1. An AE will be assumed to be treatment-emergent if non-treatment emergence cannot be reasonably ruled out. In order to reflect the adverse event profile with routine prophylaxis treatment, summaries of TEAEs will exclude AEs during the surgical period and subcutaneous substudy. TEAEs during those periods will be presented separately. TEAEs will be summarized separately for subjects in PUPs and PTPs.

Adverse events will be recorded from the time when informed consent is granted at the start of this extension study until study completion (or the end of study visit upon early termination). In this study, treatment-emergent AEs are defined as those with onset on or after Day 1 in this study.

Related AEs are AEs considered by the investigator to be related to study treatment.

TEAEs leading to withdrawal from study or withdrawal from study medication are any TEAEs reported on the AE log with "Was subject terminated from study due to the AE" as "Yes" or "Action taken with study drug" with response of "Permanently discontinued" respectively.

Adverse events of special interest (AESI) will include preferred terms associated with the following Standard MedDRA Queries (SMQs) based on narrow search criteria:

1. Anaphylactic reactions
2. Embolic and thrombotic reactions
3. Hypersensitivity

AEs will be reported separately for the surgery substudy, subcutaneous substudy, main study (prophylaxis and PK period), and overall (all periods). The following will be summarized by SOC and PT for the number and percentage of subjects who experience at least 1 event and the total number of events:

- all AEs including AESIs
- all SAEs
- TEAEs, overall and by regimen
- related TEAEs
- TEAEs occurring ≤ 72h after infusion of study medication
- non-serious TEAEs
- SAEs
- related treatment-emergent SAEs
- TEAEs by maximum severity (If a subject experiences multiple events that map to a single PT, only the most severe event will be counted.)
- TEAEs by highest relationship and maximum severity (If a subject experiences multiple events that map to a single PT, only the related event and the most severe will be counted.)
- TEAEs leading to withdrawal of study medication
- TEAEs leading to death
- TEAEs by every six-month time intervals (not applicable for PUPs)

SOCs will be displayed by decreasing frequency, and PTs will be displayed in decreasing frequency by subject.

TEAEs also will be presented for the following subgroups:

- subjects ≥18 years who switched from 14-day regimen to 21-day regimen
- subjects ≥18 years who switched to 21-day regimen compared with subjects ≥12 years not switching
- subjects <12 years in 14-day regimen
    - paired subjects who had 7-day and 14-day regimen in CSL654_3003
    - subjects in CSL654_3003 14-day regimen
    - subjects in CSL654_3002 who had 14-day regimen in CSL654_3003

- subjects <12 years who switched to 14-day regimen compared with subjects <12 years not switching

TEAEs will be counted in the respective regimen based on the onset time of the TEAE.

Adverse events associated with COVID-19 will be summarized for PUPs. These events will be identified based on predefined terms from SMQs as described above. An overall summary and a summary of TEAEs by SOC and preferred term will be provided. A supportive listing of adverse events associated with COVID-19 will be included.

4.13.4 Local Tolerability

Subject assessment of tolerability will be displayed with investigator assessment of tolerability.

Subgroup analyses of local tolerability will include the subgroups listed for TEAEs in Section 4.13.3 of this SAP. The local tolerability assessments during the surgery and subcutaneous substudies will not be included. Analyses of local tolerability will exclude tolerability assessments during the surgery and subcutaneous substudies.

4.13.5 Laboratory Safety Parameters

Laboratory results along with the change from baseline will be summarized by parameter and study visit. The change from baseline to the lowest value after first dose of study product, highest value after first dose of study product, and final value will also be summarized. For laboratory parameters with positive or negative results, the number and proportion with positive treatment-emergent results will be summarized at each visit.

Laboratory values will be converted to the standard units of measurement for summary displays.

4.13.6 Antibodies to rIX-FP and CHO Cell-derived Proteins

The number and percentage of subjects with rIX-FP and CHO cell-derived protein antibody titers above the cut-off level for the respective confirmation assays will be tabulated.

4.13.7 Vital Signs

Vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], pulse rate, and temperature) results along with the change from baseline will be summarized by parameter and study visit. The number and proportion of subjects with treatment-emergent potentially clinically significant (PCS) vital sign values by study visit will be tabulated for PTPs. Criteria for PCS values will be derived in the analysis dataset and summarized by study visit according to the criteria in Table 8.

Table 8. Criteria for Potentially Clinically Significant Vital Sign Results

Vital Sign	Criteria	Definition of PCS
Systolic Blood Pressure	Low	values ≤90 mmHg and decreased ≥20 mmHg from initial value
	High	values ≥180 mmHg and increased ≥20 mmHg from initial value

Diastolic Blood Pressure	Low	values ≤50 mmHg and decreased ≥15 mmHg from initial value
	High	values ≥105 mmHg and increased ≥15 mmHg from initial value
Heart Rate	Low	values ≤50 bpm and decreased ≥15 bpm from initial value
	High	values ≥120 bpm and increased ≥15 bpm from initial value

4.13.8 Virus Safety

Results from virus safety samples will be listed.

4.13.9 Additional Safety Information from the Surgery Substudy

Safety assessments and events, including AEs that occur during the preoperative, intraoperative, or postoperative periods of the surgery substudy will be summarized in separate tables (Section 6.7.7).

4.13.10 Additional Safety Information from the Subcutaneous Substudy

Safety assessments and events, including AEs that occur during the SC substudy will be summarized in separate tables (Section 7).

4.14 Pharmacokinetics and Pharmacodynamics Data

4.14.1 Pharmacokinetics

A non-compartmental analysis (NCA) will be performed on plasma FIX activity using WinNonlin® 6.2.1 or higher. The PK parameters will be derived with and without correction for baseline FIX levels. Baseline levels are defined as the pre-dose levels before a specific product administration. Descriptive statistics will be presented for both baseline-corrected and uncorrected NCA PK parameters. In the event that considerable residual levels of previous product (e.g. due to prior rIX-FP dosing) are evident at pre-dose, calculations of baseline-corrected PK parameters (by subtraction of the pre-dose FIX activity level from subsequent post-dose levels) may be limited to incremental recovery (IR), C_{max}, and t_{max}.

Incremental recovery will be calculated using the PK samples obtained following injection of 100 IU/kg rIX-FP for PTPs or 50 IU/kg rIX-FP for PUPs. Incremental recovery (IU/mL/IU/kg) is defined as FIX activity (IU/mL) obtained 30 minutes following injection, per dose of (IU/kg) injection. IR and FIX activity levels will be summarized.

The PK endpoints are:

- Observed trough (pre-dose) and steady-state PK FIX activity.
- PK parameters: IR, C_{max}, t_{max}, AUC_{last}, $AUC_{0-\infty}$, AUC_{ext}, λ_z , λ_z (Lower), λ_z (Upper), $t_{½}$, CL, CL/BW, Vz, Vz/BW, Vss, Vss/BW, MRT and $AUMC_{0-\infty}$.

Population PK modeling and simulation will also be performed. A separate Modeling Analysis Plan (MAP) will further define the population PK analysis, parameters and simulation methods, and these model results will be summarized in a separate supplemental report.

FIX activity levels from central and local laboratories will be summarized with descriptive statistics and listed by subject and assessment time point. Trough levels will be summarized by regimen.

Trough and steady-state flags will be derived for the observed PK FIX activity.

The following FIX results will be excluded from the derivation of the trough FIX:

- Any FIX activity measured at a local lab
- Any FIX activity measured during PK, repeated PK, surgical and subcutaneous periods
- Any FIX activity measured during an unscheduled visit
- If there are any additional doses administered (such as treating a bleeding event or additional dose) between 2 prophylaxis dose time intervals, the FIX activity measured before the second prophylaxis dose will be excluded.

Steady-state FIX is the subset of trough FIX. Two additional conditions will be applied to trough FIX values to derive the steady-state FIX:

- FIX trough samples that are not after the following will be excluded
    - 1 consecutive dose on 21-day regimen or
    - 2 consecutive doses on the 14-day or 10-day regimens or
    - 3 consecutive doses on the 7-day regimen
- If additional FIX product is administered (e.g. rescue), the subsequent trough FIX value occurring within 21 days for the 21-day regimen, 28 days for 14-day or 10-day regimens, or 21 days for the 7-day regimen will be excluded as the washout period for rIX-FP is about 5 half-lives.

Descriptive statistics for the observed trough and steady state PK FIX results will be provided for all prophylaxis regimens overall and by age (Section 4.1.1)

4.14.2 Additional Pharmacokinetic Information from the Subcutaneous Substudy

The PK assessments of rIX-FP which occur during the PK period of the subcutaneous substudy will be summarized in separate tables (Section 7.6.2).

4.14.3 Pharmacodynamics

Not applicable.

4.15 Other Analyses

Not applicable.

5 Subset Analyses of Japanese Subjects

Subset analyses will be performed for Japanese subjects. All analyses of Japanese subjects will be based on the subjects who were enrolled in Japan.

Summaries from the main study as applicable will be repeated for the Japanese subset of subjects for the following:

- subject disposition
- protocol deviations
- demographics and baseline characteristics
- prior on concomitant Medications
- exposure and time on study
- compliance
- ABRs for each bleeding event type
- consumption during routine prophylaxis
- investigator's assessment of hemostatic efficacy
- number of bleeding events
- FIX activity concentrations
- uncorrected and baseline-corrected FIX activity PK parameters following administration of rIX-FP by dose
- FIX activity trough and steady state
- TEAEs (summary and by SOC and PT) as follows:
  - TEAEs
  - all SAEs.
  - related TEAEs
  - related treatment-emergent SAEs
  - TEAEs by maximum severity. If a subject experiences multiple events that map to a single PT, only the most severe event will be counted.
  - TEAEs by relationship. If a subject experiences multiple events that map to a single PT, only the related event will be counted.
  - TEAEs occurring ≤ 72 h after infusion
  - TEAEs leading to withdrawal of study medication and by regimen
  - TEAEs due to death
  - TEAEs occurring in ≥2% of subjects
  - TEAE by every 6-month interval
- local tolerability

- inhibitors and antibodies to FIX
- laboratory results – hematology and biochemistry
- vital signs
- non-pharmacologic interventions

Analyses of exposure, TEAEs, and local tolerability will be presented for the Japanese subjects ≥18 years of age who switched from the 14-day regimen to the 21-day regimen in Study CSL654_3003 and for Japanese subjects ≥12 years of age who did not switch.

The following analyses of ABR and AsBR will be presented for Japanese subjects:

- subjects ≥18 years in both the 21-day and 7-day regimens
- subjects ≥18 years in both the 21-day and 14-day regimens

No hypothesis tests will be presented for the Japanese subset due to the limited number of Japanese subjects in each regimen. Point estimates and 95% CIs will be presented if estimable.

Separate listings for Japanese subjects in the main study will not be produced as all listings for the study will include country and race. Analyses of Japanese subjects in the surgery substudy will be presented in subject listings. No Japanese subjects participated in the SC substudy.

Figures for PK/FIX activity for Japanese subjects will be provided if applicable (i.e. number of Japanese subjects with FIX activity based on the 50 and 100 IU/kg doses).

6 Surgery Substudy

6.1 Introduction

This substudy of Study CSL654_3003 will examine the efficacy of rIX-FP in subjects with severe hemophilia B who are undergoing non-emergency major or minor surgery. The efficacy endpoint, investigator's (or surgeon's) overall clinical assessment of hemostatic efficacy, will be measured during surgical periods. The safety endpoints, including antibodies against rIX-FP, inhibitors to FIX, and other AEs throughout the duration of the study will be provided.

The substudy will be conducted in compliance with this study protocol, ICH Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

6.2 STUDY OBJECTIVES AND ENDPOINTS

Surgery Substudy Primary Objective and Endpoint

Primary Objective

The primary objective of the substudy is to evaluate the efficacy of rIX-FP in the prevention and control of bleeding in subjects with severe hemophilia B during surgical procedures.

Primary Endpoint

Investigator's (or surgeon's) overall clinical assessment of hemostatic efficacy for surgical prophylaxis, based on a four point ordinal scale (excellent, good, moderate, poor / none)

Surgery Substudy Secondary Objectives and Endpoints

Secondary Objective

The secondary objective of the study is to evaluate the safety of rIX-FP during the intraoperative and postoperative periods.

Secondary Endpoints

The secondary endpoints of this surgery substudy are:

- TEAEs
- The frequency of AEs related to rIX-FP during the intraoperative and postoperative periods.
- The occurrence of FIX inhibitors.
- The occurrence of antibodies against rIX-FP.
- Comparison of predicted and intraoperative estimated blood loss.
- Comparison of predicted and actual transfusion requirements.
- Change in hemoglobin levels from baseline, intraoperatively and postoperatively.

6.3 Discussion of Surgery STUDY DESIGN

This is a prospective, open-label substudy to evaluate the efficacy and safety of rIX-FP to prevent bleeding during surgery in subjects with congenital FIX deficiency (hemophilia B). The study will consist of intraoperative and postoperative safety and efficacy evaluation periods with rIX-FP. Major surgery is defined as a surgical procedure that involves anesthesia (general, spinal, epidural or regional block), respiratory assistance, or requires hemostatic therapy for periods exceeding 5 consecutive days (including but not limited to orthopedic and cardiac surgery).

Approximately 3 hours before the non-emergency surgical procedure, the subject will receive a single bolus injection of rIX-FP in order to increase the plasma FIX activity level to either 60% to 80%, or higher, as recommended by the WFH for major surgery, or higher if recommended by the guidelines of the local hospital / practice. Additional bolus injections of rIX-FP may be administered during a major surgery, if needed. Blood samples for the determination of FIX levels will be collected before administration of additional doses of rIX-FP, if feasible. During the postoperative period, additional bolus injections of rIX-FP may be administered to maintain a required trough FIX activity level as recommended by the WFH at the investigator's discretion.

Further details of the surgery substudy design are provided in the CSL654_3003 Protocol, Appendix 1. Additional definitions related to the surgery substudy are provided below:

- Minor Surgery is defined as any non-emergency surgery that is not a major surgery.
- Baseline in the surgery substudy is defined as last assessment performed up to 28 days before surgery.
- Intraoperative period will be defined as during surgery.
- Postoperative period is defined as after surgery up to 28 days.

Number of Subjects

Any subjects requiring surgery during the course of the main study (CSL654_3003) will be enrolled in the surgery substudy, with a combined target enrollment of at least 5 subjects and 10 major surgeries across the lead-in studies and this study.

Duration

The substudy design includes a 1- to 14-day (or longer, if needed) active treatment period. Subjects will then return to the treatment plan of the main study at Day 28 ± 7 days. The duration of the active treatment period will depend on the type of surgery and local standard of care.

Eligibility: Selection of Substudy Population

For this substudy, subjects must meet all eligibility criteria of the main study defined in the protocol and must:

- require non-emergency surgery and
- provide written informed consent for substudy participation.

6.4 ALLOCATION, DOSING AND ADMINISTRATION

For detailed discussion please refer to Protocol CSL654_3003 Appendix 1.

Concomitant Therapy

For a detailed discussion refer to Section 4.9.

6.5 VISIT SCHEDULE

The schedule of assessments for subjects undergoing major surgery is provided in Figure 6, and the schedule of assessment for subjects undergoing minor surgery is provided in Figure 7.

Figure 6. Schedule of Assessments: Surgery Substudy – Major Surgery

Period	Before Surgery			During Surgery	After Surgery		
Timing		-3 hours		N/A	0 hours	Every 24 hours up to 72 hours or discharge [A]	End of Substudy Day 28 [B]
Time Window	Up to 28 days before	-6 hours			+6 hours	± 6 hours	± 7 days
Eligibility assessment		X					
Surgery description		X					
Physical examination		X			X	X	X
Vital signs		X		X	X	X	X
Body weight		X					
Hematology [C]		X		X	X	X	X
Plasma FIX activity [D]	X [E]		X [F]	X [G]	X [G]	X [G]	X
FIX inhibitors [H]	X						X
antibodies against rIX-FP [H]	X						X
Planned rIX-FP dose	X						
Blood loss estimate	X				X		
Hemostatic intervention [I]	X			X	X	X	
Administer rIX-FP [J]		X		X [G]	X [G]	X [G]	
Hemostasis assessment [K]					X	X	
Efficacy assessment					X	X	
Adverse events	X	<----------On an ongoing basis---------->					
Concomitant therapy	X	<----------On an ongoing basis---------->					

FIX = coagulation factor IX; N/A = not applicable; rIX-FP = recombinant coagulation factor IX albumin fusion protein.

Notes to the Schedule of Assessments: Surgery Substudy – Major Surgery

A: Assessments to be completed every 24 hours up to 72 hours or up to hospital discharge, whichever occurs first.
B: Assessments to be completed at end of surgery substudy for all subjects (Day 28). This visit is also the Day 1 visit for subjects in Arm 3, if they choose to remain in the main study after surgery.
C: Hematology assessments (local laboratory) include: hemoglobin, hematocrit, platelet count, red blood cell (erythrocyte) count, white blood cell (leukocyte) count, white blood cell differential (optional).
D: Plasma FIX activity will be assessed by both the local laboratory and a central laboratory.
E: Assessment is not required if the FIX recovery rate has been assessed within the previous 28 days or for subjects in Arm 3.
F: Blood samples will be taken between ~30 minutes after the administration of rIX-FP and before major surgery (timing dependent on the time required for the local laboratory to process the sample).
G: If an additional injection of rIX-FP is needed during the perioperative period, a blood sample should be taken before the administration of rIX-FP for the assessment of FIX activity by the local laboratory.
H: To be completed only for subjects in Arms 1 or 2.
I: Planned and actual hemostatic interventions or transfusions other than rIX-FP (eg, whole blood, red blood cells, fresh frozen plasma or platelets).
J: Additional injections of rIX-FP may be needed to maintain the required plasma FIX activity level. Blood samples should be taken and tested for plasma FIX activity before each injection of rIX-FP, if feasible, so that the required dose can be calculated based on the plasma FIX activity.
K: Hemostasis assessment includes: quantitation of postoperative bleeding through surgical drains and the occurrence of late rebleeding episodes; the presence and extent of any surgical wound hematomas and whether they require surgical evacuation.

Figure 7. Schedule of Assessments: Minor Surgery

Period	Before Surgery			During Surgery	After Surgery		
Timing	-3 hours			N/A	0 hours	At Discharge	End of Substudy
Time Window	Up to -6 hours				+6 hours	± 6 hours	
Eligibility assessment	X						
Surgery description	X						
Physical examination					X	X	X
Vital signs					X	X	X
Body weight	X						
Plasma FIX activity [A]	X [B]		X [B]	X [C]	X [C]	X [C]	X
Sample for FIX inhibitors							X
Sample for antibodies against rIX-FP							X
Blood loss estimate			X		X		
Hemostatic intervention [D]			X	X			
Planned rIX-FP dose during surgery			X				
Administer rIX-FP		X		X [C]	X [C]	X [C]	
Hemostasis assessment [E]					X	X	
Efficacy assessment					X	X	
Adverse events	X	<----------On an ongoing basis---------->					
Concomitant therapy	X	<----------On an ongoing basis---------->					

FIX = coagulation factor IX; N/A = not applicable; rIX-FP = recombinant coagulation factor IX albumin fusion protein.

Notes to the Schedule of Assessments: Surgery Substudy – Minor Surgery
A: Plasma FIX activity may be assessed by the local laboratory and / or the central laboratory.
B: Blood samples (optional) may be taken before and ~30 minutes after rIX-FP administration to confirm the incremental recovery.
C: Additional injections of rIX-FP may be needed to maintain the required plasma FIX activity level. Blood samples should be taken and tested for plasma FIX activity before each injection of rIX-FP, if feasible, so that the required dose can be calculated based on the plasma FIX activity.
D: Planned and actual hemostatic interventions or transfusions other than rIX-FP (eg, whole blood, red blood cells, fresh frozen plasma or platelets.
E: Hemostasis assessment includes estimated actual intraoperative blood loss, blood loss expected as integral to the procedure and unexpected blood loss due to unforeseen complications.

End of Participation in the Surgery Substudy (Day 28)

The End of Substudy visit should be conducted at Day 28 after last surgery (in the event that an additional surgery is performed) (see CSL654_3003 Protocol Appendix 1 Section C) and routine prophylaxis therapy with rIX-FP should be started (subjects in Arm 3) or restarted (subjects in Arms 1 or 2).

6.6 STUDY VARIABLES AND METHODS OF ASSESSMENT

Details of the Surgery substudy are provided in Protocol CSL654_3003Appendix 1.

Subject Characteristics

The following subject characteristics will be collected for the surgical population:

- demographics
- medical and surgical history
- treatment history (rIX-FP or other FIX product)

Surgery Substudy Efficacy Variables

Overview of Variables

The primary clinical efficacy parameters assessed in the study are study product usage and hemostasis assessments by the Investigator.

Surgery Substudy ***Primary efficacy variable:***

Investigator's evaluation of efficacy of rIX-FP prophylaxis during surgery (Table 9)

Surgery Substudy ***Secondary efficacy variables****:*

- comparison of predicted and estimated intraoperative blood loss.
- comparison of predicted and actual transfusion requirements.
- change in hemoglobin levels between baseline, intra-operation and post-operation.

Methods of Assessment

Perioperative Prophylaxis for Surgeries

The investigator will document the following:

- type of surgery
- relationship to hemophilia: related/not related
- dose of rIX-FP
- preoperative predicted blood loss (average and maximum) for a non-hemophilic individual undergoing the same type and extent of surgical procedure
- predicted hemostatic intervention, other than rIX-FP and transfusion requirements (if any), for a non-hemophilic individual undergoing the same type and extent of surgical procedure
- actual use of additional hemostatic interventions other than rIX-FP and transfusion blood products following the start of injection with rIX-FP (including blood products and other coagulation factor[s])
- the estimated intraoperative blood loss
- the presence and extent of any surgical wound hematomas noting whether they require surgical evacuation
- quantification of postoperative bleeding through surgical drains and the occurrence of late bleeding events
- hemoglobin levels at baseline and nadir-, intra-, and post-operation

Table 9. Investigator's Evaluation of Efficacy of Surgical Treatment

Excellent	Hemostasis clinically not significantly different from normal (e.g., achieved hemostasis comparable to that expected during similar surgery in a non factor-deficient patient) in the absence of other (unplanned) hemostatic intervention or estimated actual blood loss during surgery is not more than 20% higher than the estimated predicted blood loss for the intended surgery.
Good	Normal or mildly abnormal hemostasis in terms of quantity and/or quality (e.g., slight oozing, prolonged time to hemostasis with somewhat increased bleeding compared to a non-factor deficient patient in the absence of other [unplanned] hemostatic intervention) or estimated actual blood loss is greater than 20% but less than or equal to 30% higher than the estimated predicted blood loss for intended surgery.
Moderate	Moderately abnormal hemostasis in terms of quantity and/or quality (e.g., 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 (e.g., severe hemorrhage that is difficult to control) and/or additional hemostatic intervention required with another factor IX product for complete resolution.

Surgery Substudy Safety Variables

Overview of Variables

Safety will be assessed on the basis of the following variables:

- AEs including SAEs and AESIs.
- laboratory safety parameters (blood tests).
- vital signs.
- physical examination.
- antibodies against rIX-FP.
- inhibitors against FIX.

Methods of Assessment

See Section 4.12 for details.

6.7 STATISTICS

6.7.1 Determination of Sample Size

The number of subjects in the surgery substudy is not powered for statistical significance.

6.7.2 Analysis Populations

Surgical population

See Section 3.6.7 for details.

6.7.3 Statistical Analyses and Methods

All summaries and analyses will be based only on the subjects in this substudy. Continuous data will be summarized using descriptive statistics including means, SD, medians, first and third quartiles, and minimums and maximums. Categorical variables will be summarized with frequencies and percentages.

Unless otherwise indicated, baseline is defined as the last measurement obtained before the start of the intraoperative period.

No hypothesis tests will be presented for the surgery substudy.

Subjects with multiple surgeries will be counted for each surgery evaluated in this substudy.

6.7.4 Disposition, Demographics, and Baseline Characteristics

Disposition, including subjects who completed or withdrew from the surgery substudy, number of major and minor surgeries (including whether or not related to hemophilia), will be summarized for all subjects in the Surgical population. Demographics, medical and surgical history, and treatment history will be summarized for the surgical population.

6.7.5 Concomitant Medications

Summaries of concomitant medications will include the preferred drug name within the ATC level 4 classification. The summaries will be presented for the surgical population. If a subject has multiple occurrences of a medication, then the subject will be presented only once in the respective subject count.

6.7.6 Efficacy

6.7.6.1 Investigator's Overall Clinical Assessment of Hemostatic Efficacy for Surgical Prophylaxis

The assessment categories in Table 9 will be tabulated and presented by time point for all surgeries in the surgical population.

6.7.6.2 Consumption of rIX-FP during Surgery Substudy

Consumption of rIX-FP (IU/kg) will be summarized with descriptive statistics at the following timepoints:

- total pre-surgery

- total intraoperative period
- post-surgery: 0-72 hours, 72-≤168 hours, 168-≤336 hours, 336-≤504 hours, 504-≤672 hours
- Overall

6.7.7 Safety

6.7.7.1 Treatment-Emergent Adverse Events during the Surgery Substudy

TEAEs (Section 4.13.3) will be summarized by surgical period (i.e. overall, intraoperative and post-operative) according to the onset date of the TEAE. The following TEAEs will be summarized by SOC and PT separately for the intraoperative period and the post-operative period:

- all TEAEs
- related TEAEs
- TEAEs leading to withdrawal of study medication
- SAEs
- related SAEs

6.7.7.2 Additional Safety Assessments

Inhibitors and Antibodies

The number and percentage of subjects with inhibitors against FIX (Section 4.13.2) and 95% exact Clopper-Pearson CI will be presented as applicable for all subjects in the surgical population. The number and percentage of subjects with positive or negative results to the following antibodies also will be included:

- rIX-FP
- pdFIX
- rFIX
- albumin
- antibodies against CHO cells

Change in Hemoglobin

The absolute value and change in hemoglobin from the last pre-operative measurement to the intraoperative and post-operative measurements (0, 24, 48, 72 hours, and end of surgical substudy) will be summarized with descriptive statistics. Descriptive statistics for the lowest intraoperative and post-operative values also will be included.

Change in Vital Signs

The absolute value and change in vital signs (i.e. SBP, DBP, heart rate, and temperature) from the last pre-operative measurement to the intraoperative and post-operative

measurements (0, 24, 48, 72 hours, and end of surgery substudy) will be summarized with descriptive statistics. Descriptive statistics for the lowest intraoperative and post-operative values also will be included.

Comparison of Predicted and Intraoperative Estimated Blood Loss during Surgery

Descriptive statistics will be provided for the average and maximum predicted blood loss (mL) and estimated actual blood loss during the surgery substudy.

Comparison of Predicted and Actual Transfusion Requirements during Surgery

Descriptive statistics will be provided for the predicted and actual units of packed red blood cells, whole blood, and any other transfusion products utilized during surgery.

7 Subcutaneous Substudy

7.1 Introduction

This substudy of CSL654_3003 will evaluate the safety and PK of single and repeated SC administration of rIX-FP in adults with severe hemophilia B (i.e., FIX activity of ≤ 2%) who are currently enrolled in the study. Safety will be measured by the frequencies of AEs, SAEs, investigator and subject assessments of local tolerability, and the incidence of inhibitors to rIX-FP. Pharmacokinetic endpoints will be measured based on FIX activity.

Subjects enrolled in the SC substudy will be sequentially assigned to a dose cohort (Cohort 1 [single dose of 25 IU/kg], Cohort 2 [single dose of 50 IU/kg], Cohort 3 [15 doses of 25 IU/kg every 3 days], and an optional Cohort 4 [15 doses of ≤ 50 IU/kg every 5 days, with the actual dose in this cohort to be determined by CSL based on the PK results from Cohort 3]).

The substudy will be conducted in compliance with this study protocol, ICH GCP, and the applicable regulatory requirement(s).

7.2 STUDY OBJECTIVES AND ENDPOINTS

CCI

7.3 Discussion of Subcutaneous Substudy Study Design

For a detailed discussion refer to Protocol CSL654_3003 Appendix 2.

Number of Subjects

This substudy will enroll approximately 4 to 6 male subjects each in Cohorts 1 to 4 (i.e., up to a maximum of 24 subjects). Subjects may enroll in more than 1 cohort.

Duration

The maximum total substudy duration of each subject will be approximately 30 days for subjects in Cohorts 1 and 2, approximately 57 days for subjects in Cohort 3, and approximately 85 days for subjects in Cohort 4. Subjects will then return to the IV treatment plan of the main study.

The overall substudy duration (i.e., first subject's Day 1 visit to the last subject's end of substudy visit) is expected to be 18 to 22 months and includes estimated durations for screening, enrollment of the subjects into the substudy, and the final visit.

Eligibility: Selection of Substudy Population

Subjects meeting all of the following inclusion criteria may be enrolled into the substudy:

1. Male subjects, at least 18 years of age.
2. Subjects who are currently enrolled in Study CSL654_3003 with hemophilia B (FIX activity ≤ 2%).
3. Subjects who have received rIX-FP for ≥ 100 EDs (Cohorts 1 and 2) or for ≥ 50 EDs (Cohorts 3 and 4).
4. Body weight between 50.0 and 100.0 kg, both inclusive.
5. Body mass index (BMI) between 18.0 and 29.9 kg/m^2.

6. Written informed consent for substudy participation obtained before undergoing any specific procedures.

Exclusion Criteria

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

1. Use of rIX-FP within 14 days of SC administration of rIX-FP.
2. Suspected inability (e.g., language problems) or unwillingness to comply with study procedures.
3. Experienced a life-threatening bleeding event or had major surgery during the 3 months prior to substudy entry (Day 1).

7.4 ALLOCATION, DOSING AND ADMINISTRATION

For a detailed discussion refer to Protocol CSL654_3003, Appendix 2.

7.5 VISIT SCHEDULE

Procedures to be performed before and after subcutaneous administration are provided in the Schedule of Assessments in Protocol CSL654_3003, Appendix 2. The schedule of assessments for Cohorts 1 and 2 are provided in Figure 8. The schedules of assessments for Cohort 3 are provided in Figure 9 and Figure 10. The schedules of assessments for Cohort 4 are provided in Figure 11 and Figure 12.

Figure 8. Schedule of Assessments for Cohorts 1 and 2: Subcutaneous Administration Substudy Screening, PK Assessment, and Safety Follow-Up

	Screening	Active Treatment Period											PK & Safety Follow-up	
Substudy Day		Day 1 [A] (before SC dose)	Day 1	Day 1 (after SC dose)	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 11	Day 15 [B]	Day 15	Day 28
Assessment Time Point			0 h	0.5 h	3 h	24 h	48 h	72 h	120 h	168 h	240 h	336 h	0.5 h	
Assessment Time Window	1 to -28 d			± 10 m	± 30 m	± 3 h	± 6 h	± 6 h	± 6 h	± 6 h	± 6 h	± 6 h	± 10 m	± 4 d
Informed consent	X													
Eligibility assessment	X	X												
Physical examination		X										X		X
Vital signs [C]		X		X								X		X
Plasma FIX activity (CL, optional LL)[D]		X		X	X	X	X	X	X	X	X	X	X	X
Inhibitors against FIX (CL)		X										X		X
Antibodies against rIX-FP and CHO cells (CL)[E]		X										X		X
Activation of coagulation tests (CL)[F]		X		X	X	X								
SC dosing of rIX-FP [G]			X											
IVdosing of rIX-FP IV												X [H]		
Adverse events		< ----------On an Ongoing Basis---------- >												
Concomitant therapies		< ----------On an Ongoing Basis---------- >												

CHO = Chinese hamster ovary; CL = central laboratory; d = day; FIX = coagulation factor IX; IU = international unit; IV = intravenous; LL = local laboratory; m = minutes; rIX-FP = recombinant coagulation factor IX albumin fusion protein; SC = subcutaneous; TAT = thrombin-antithrombin.

Notes to the Schedule of Assessments for Cohorts 1 and 2:

A: Subjects are considered to be in the main study until Day 1 of the substudy.
B: Day 15 assessments, including administration of IV rIX-FP, may occur earlier if the LL FIX activity is ≤ 2 IU/dL.
C: Vital signs include measurement of body weight. Weight only measured Day 1 (prior to dose), Day 15 and Day 28.
D: Blood PK samples are at predose and at 0.5, 3, 24, 48, 72, 120, 168, 240 and 336 hours after the start of SC administration; at predose and at 0.5 hours post IV administration and at End of Substudy (Day 28). Samples will be tested by the central laboratory; however, the site may request to have additional samples tested by the local laboratory (LL). If LL FIX activity is ≤ 2 IU/dL, at any time point, subsequent time points may be omitted and the IV dose may be administered. If LL FIX activity is > 5 IU/dL at 336 hours, up to 3 additional blood PK samples may be taken every 48 hours (384, 432, and 480 hours) until LL is < 5 IU/dL, and the IV dose delayed until completion of the additional PK sampling at the discretion of the investigator.
E: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between plasma-derived FIX, recombinant FIX and albumin antibodies.
F: Blood samples for activation of coagulation tests include D-dimer, TAT, and prothrombin F1+2.
G: SC rIX-FP should be administered at least 14 days after the previous IV rIX-FP administration (21 days if subject is on a 21-day prophylaxis regimen).
H: Intravenous administration of rIX-FP will occur after the Day 15 blood draws.

Figure 9. Schedule of Assessments for Cohort 3: Dosing and Visit Schedule

Substudy Day for Cohort 3 [A]	Screening	Dose 1	Dose 2	Dose 3	Doses 4 to 12; Dose 14	Dose 13	Dose 15	PK & Safety Follow-up [C]	
		Day 1 [B]	Days 2 to 4	Day 7	Days 10 to 34; Day 40	Day 37	Day 43	Days 44, 45, 46, 47, and 53	Day 57 (EOS)
Time Windows	1 to -28 days	See "Cohort 3 Sampling Schedule" for details				See "Cohort 3 Sampling Schedule" for details			
Informed consent	X								
Eligibility assessment	X	X							
Physical examination		X							X
Vital signs		X [D]					X [D]		X [D]
Predose plasma FIX activity (CL)		X		X		X	X		
Postdose plasma FIX activity (CL) [E]		X	X				X	X	X
Inhibitors against FIX (CL)		X [F]							X [F]
Antibodies against rIX-FP and CHO cells (CL)		X [F]							X [F]
Activation of coagulation tests (CL) [E]		X	X				X	X	X
SC dosing of rIX-FP at study site		X [G]	X	X		X	X		
Training for SC home administration				X					
Dispense IMP [H]				X		X			
SC dosing of rIX-FP at home					X				
eDiary review			X	X		X	X	X	X
IMP return/accountability						X	X		
Investigator assessment of local tolerability [I]		X	X	X		X	X	X	X
Subject assessment of local tolerability					X [J]				
Adverse events		< ----------On an Ongoing Basis---------->							
Concomitant therapies		< ----------On an Ongoing Basis---------->							

CHO = Chinese hamster ovary; CL = central laboratory; EOS = end of substudy; FIX = coagulation factor IX; IU = international unit; IV = intravenous; rIX-FP = recombinant coagulation factor IX albumin fusion protein; PK = pharmacokinetics; SC = subcutaneous; TAT = thrombin-antithrombin.

Notes to the Schedule of Assessments for Cohort 3:

A: Dosing schedule in Cohort 3: 25 IU/kg every 3 days. Doses 4 to 12 and Dose 14 are administered by the subject at home.
B: Subjects are considered to be in the main study until Day 1 of the substudy.
C: For subjects who discontinue before SC Dose 15, the site should perform the serial PK assessments after the subject's final SC dose, if possible. If this is not possible, at a minimum an EOS PK sample should be taken.
D: SBP, DBP, heart rate, and body temperature assessed before dose and at 30 min after dose. In addition, body weight and height at Day 1 (before dose), and body weight at the EOS visit (336 hours after final SC dose).
E: See the "Cohort 3 Sampling Schedule" for details on the sampling time points and time windows.
F: Blood samples for inhibitors against FIX and for antibodies against rIX-FP and CHO cells must be taken a) before the first SC dose and b) after the final SC dose but before the subject returns to IV treatment in the main study. See the "Cohort 3 Sampling Schedule" for details.
G: First rIX-FP SC dose should be administered at least 14 days after the previous rIX-FP IV administration in the main study (21 days if subject is on a 21-day prophylaxis regimen).
H: Includes IMP for SC home administration and for on-demand IV treatment of any bleeding episodes that may occur during the SC home administration periods.
I: See the "Cohort 3 Sampling Schedule" for the time points of local tolerability assessment by the investigator.
J: Subjects will record their overall perception of local tolerability in the eDiary at 0.5, 8, and 24 hours after each SC injection at home.

Figure 10. Subcutaneous Substudy – Cohort 3 Sampling Schedule and Investigator Local Tolerability Assessments for SC Doses Administered at Study Site

SC Dose [A]	1					2	3 & 13	15			PK & Safety Follow-up [D]					
Substudy Day [A]	1			2	3	4	7 & 37	43			44	45	46	47	53	57
Time Point	Pre [B]	0.5 h	3 & 8 h	24 h	48 h	72 h [C]	Pre	Pre	0.5 h	3 & 8 h	24 h	48 h	72 h	96 h	240 h	336 h
Time Windows		±10 m	±30 m	±3 h	±24 h	±6 h	±24 h	±24 h	±10 m	±30 m	±3 h	±6 h	±6 h	±6 h	±6 h	±6 h
Plasma FIX activity (CL)	X	X	X	X	X	X [E]	X [E]	X [E]	X	X	X	X	X	X	X	X
Inhibitors against FIX (CL)	X															X
Antibodies against rIX-FP and CHO cells (CL) [F]	X															X
Activation of coagulation tests (CL) [G]	X	X	X	X				X	X	X	X					X
Investigator assessment of local tolerability		X	X	X	X	X [H]	X [H]		X	X	X	X	X	X	X	X

CHO = Chinese hamster ovary; CL = central laboratory; FIX = coagulation factor IX; IU = international unit; IV = intravenous; rIX-FP = recombinant coagulation factor IX albumin fusion protein; PK = pharmacokinetics; SC = subcutaneous; TAT = thrombin-antithrombin.

Notes to the Cohort 3 Sampling Schedule:

A: Doses 4 to 12 and Dose 14 are administered by the subject at home (see "Schedule of Assessments for Cohort 3 – Dosing and Visit Schedule").
B: Pre = predose PK sample
C: The 72-h FIX activity sample represents the predose sample for Dose 2.
D: For subjects who discontinue from the SC substudy before SC Dose 15, the site should perform the serial PK assessments after the subject'sfinal SC dose, if possible. If this is not possible, at a minimum an EOS PK sample should be taken.
E: This plasma FIX activity sample must be taken BEFORE the dose given at these visits.
F: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between plasma-derived FIX, recombinant FIX and albumin antibodies.
G: Blood samples for activation of coagulation tests include D-dimer, TAT, and prothrombin F1+2.
H: Local tolerability assessment at 30 min after Doses 2, 3, and 13.

Figure 11. Subcutaneous Substudy – Schedule of Assessments for Cohort 4: Dosing and Visit Schedule

Substudy Day for Cohort 4 [A]	Screening	Dose 1 Day 1 [B]	Dose 2 Days 2, 3, 4, 6	Dose 3 Day 11	Doses 4 to 12; Dose 14 Days 16 to 56; Day 66	Dose 13 Day 61	Dose 15 Day 71	PK & Safety Follow-up [C] Days 72, 73, 74, 75, 81	PK & Safety Follow-up [C] Day 85 (EOS)
Time Windows	1 to -28 days	See "Cohort 4 Sampling Schedule" for details				See "Cohort 4 Sampling Schedule" for details			
Informed consent	X								
Eligibility assessment	X	X							
Physical examination		X							X
Vital signs		X [D]					X [D]		X [D]
Predose plasma FIX activity (CL)		X		X		X	X		
Postdose plasma FIX activity (CL) [E]		X	X				X	X	X
Inhibitors against FIX (CL)		X [F]							X [F]
Antibodies against rIX-FP and CHO cells (CL)		X [F]							X [F]
Activation of coagulation tests (CL) [E]		X	X				X	X	X
SC dosing of rIX-FP at study site		X [G]	X	X		X	X		
Training for SC home administration				X					
Dispense IMP [H]				X		X			
SC dosing of rIX-FP at home					X				
eDiary review			X	X		X	X	X	X
IMP return/accountability						X	X		
Investigator assessment of local tolerability [I]		X	X	X		X	X	X	X
Subject assessment of local tolerability					X [J]				
Adverse events		< ----------On an Ongoing Basis---------->							
Concomitant therapies		< ----------On an Ongoing Basis---------->							

CHO = Chinese hamster ovary; CL = central laboratory; EOS = end of substudy; FIX = coagulation factor IX; IU = international unit; IV = intravenous; rIX-FP = recombinant coagulation factor IX albumin fusion protein; PK = pharmacokinetics; SC = subcutaneous; TAT = thrombin-antithrombin.

Notes to the Schedule of Assessments for Cohort 4:

A: Dosing schedule in Cohort 4: ≤50 IU/kg every 5 days (Sponsor will determine the actual dose based on PK results from Cohort 3). Doses 4 to 12 and Dose 14 are administered by the subject at home.

B: Subjects are considered to be in the main study until Day 1 of the substudy.

C: For subjects who discontinue before SC Dose 15, the site should perform the serial PK assessments after subject's final SC dose, if possible. If this is not possible, at a minimum an EOS PK sample should be taken.

D: SBP, DBP, heart rate, and body temperature assessed before dose and at 30 min after dose. In addition, body weight and height at Day 1 (before dose), and body weight at the EOS visit (336 hours after final SC dose).

E: See the "Cohort 4 Sampling Schedule" for details on the sampling time points and time windows.

F: Blood samples for inhibitors against FIX and for antibodies against rIX-FP and CHO cells must be taken a) before the first SC dose and b) after the final SC dose but before the subject returns to IV treatment in the main study. See the "Cohort 4 Sampling Schedule" for details.

G: First rIX-FP SC dose should be administered at least 14 days after the previous rIX-FP IV administration in the main study (21 days if subject is on a 21-day prophylaxis regimen).

H: Includes IMP for SC home administration and for on-demand IV treatment of any bleeding episodes that may occur during the SC home administration periods.

I: See the "Cohort 4 Sampling Schedule" for the time points of local tolerability assessment by the investigator.

J: Subjects will record their overall perception of local tolerability in the eDiary at 0.5, 8, and 24 hours after each SC injection at home.

Figure 12. Subcutaneous Substudy – Cohort 4 Sampling Schedule and Investigator Local Tolerability Assessment for SC Doses Administered at Study Site

SC Dose [A]	1						2	3 & 13	15			PK & Safety Follow-up [D]					
Substudy Day [A]	1			2	3	4	6	11 & 61	71			72	73	74	75	81	85
Time Point	Pre[B]	0.5 h	3 & 8 h	24 h	48 h	72 h	120 h[C]	Pre	Pre	0.5 h	3 & 8 h	24 h	48 h	72 h	96 h	240 h	336 h
Time Windows		±10 m	±30 m	±3 h	±6 h	±6 h	±24 h	±24 h	±24 h	±10 m	±30 m	±3 h	±6 h	±6 h	±6 h	±6 h	±6 h
Plasma FIX activity (CL)	X	X	X	X	X	X	X[E]	X[E]	X[E]	X	X	X	X	X	X	X	X
Inhibitors against FIX (CL)	X																X
Antibodies against rIX-FP and CHO cells (CL)[F]	X																X
Activation of coagulation tests (CL)[G]	X	X	X	X					X	X	X	X					X
Investigator assessment of local tolerability		X	X	X	X	X	X[H]	X[H]		X	X	X	X	X	X	X	X

CHO = Chinese hamster ovary; CL = central laboratory; FIX = coagulation factor IX; IU = international unit; IV = intravenous; rIX-FP = recombinant coagulation factor IX albumin fusion protein; PK = pharmacokinetics; SC = subcutaneous; TAT = thrombin-antithrombin.

Notes to the Cohort 4 Sampling Schedule:

A: Doses 4 to 12 and Dose 14 are administered by the subject at home (see "Schedule of Assessments for Cohort 4 – Dosing and Visit Schedule").
B: Pre = predose PK sample
C: The 120-h FIX activity sample represents the predose sample for Dose 2.
D: For subjects who discontinue before SC Dose 15, the site should perform the serial PK assessments after the subject's final SC dose, if possible. If this is not possible, at a minimum an EOS PK sample should be taken.
E: This plasma FIX activity sample must be taken BEFORE the dose given at these visits.
F: A sample that tests positive for antibodies against rIX-FP will be retested to discriminate between plasma-derived FIX, recombinant FIX and albumin antibodies.
G: Blood samples for activation of coagulation tests include D-dimer, TAT, and prothrombin F1+2.
H: Local tolerability assessment at 0.5 h after Doses 2, 3, and 13.

7.6 STUDY VARIABLES AND METHODS OF ASSESSMENT

For an overview of the study variables and measurement times, see the Schedule of Assessments: Subcutaneous Administration Substudy Screening, PK Assessment and Safety Follow Up (Cohorts 1 and 2); Dosing and Visit Schedule (Cohorts 3 and 4, respectively); Sampling Schedule and Investigator Local Tolerability Assessments for Dose Administered at Study Site in the Protocol CSL654_3003, Appendix 2.

7.6.1 *Subcutaneous Substudy* Subject Characteristics

See Section 4.6 for details.

7.6.2 *Subcutaneous Substudy* Pharmacokinetic Analyses

Pharmacokinetic Evaluation of rIX-FP

Cohorts 1 and 2:

If during the PK assessment period, a subject experiences a bleeding event, no further blood samples will be taken as part of PK assessment, regardless of whether the bleeding event is treated or not. The PK sampling may or may not need to be repeated.

If local lab (LL) FIX activity is ≤ 2 IU/dL, at any time point, subsequent time points may be omitted and the IV dose may be administered.

If LL FIX activity is > 5 IU/dL at 336 hours, up to 3 additional blood PK samples may be taken every 48 hours (384, 432, and 480 hours) until LL FIX activity is < 5 IU/dL, and the IV

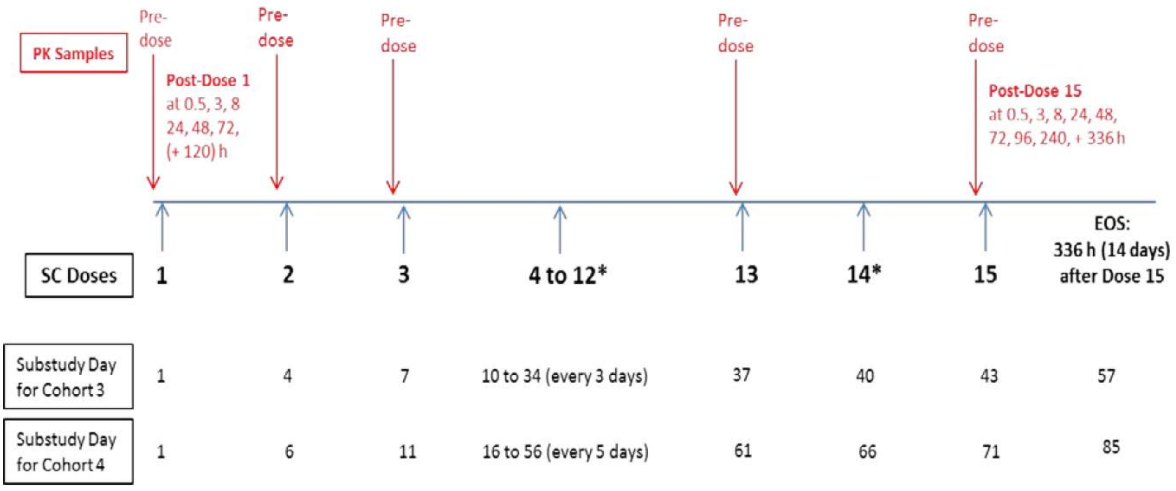
dose delayed until completion of the additional PK sampling at the discretion of the investigator.

Cohorts 3 and 4 (if applicable):

The dosing/PK sampling scheme for Cohorts 3 and 4 is provided in Figure 13.

If a subject experiences a bleeding event during the PK assessment period, the subject should treat the bleeding event with his standard on-demand IV dose of rIX-FP as prescribed in the main study. The subject should continue in the SC substudy following the same schedule of SC injections, regardless of the IV treatment of that bleeding event. All PK sampling should be completed as outlined in the Schedules of Assessments for the SC substudy. See Section 3.6 "Subcutaneous PK Population" for details on the PK inclusion of these subjects.

Figure 13. Subcutaneous Substudy: Dosing/PK Sampling Scheme and Substudy Visit Days for Cohorts 3 and 4



EOS = end of substudy; PK = pharmacokinetic; SC = subcutaneous.
Notes:
1. The 120-h sample after SC Dose 1 will only be taken in Cohort 4.
2. Doses with an asterisk will be administered by the subject/caregiver at home. All other doses will be given at the study site.

Overview of Variables

The SC pharmacokinetics of rIX-FP will be assessed on the basis of measurements of FIX level, including activity and antigen (if sufficient sample available) in plasma. PK samples will be used to measure the pharmacokinetic parameters for SC administration detailed in Table 10.

Methods of Assessment

Subcutaneous Substudy Pharmacokinetic Evaluation of rIX-FP

Blood samples for determination of FIX activity will be obtained before and after SC injection of rIX-FP at the time points detailed in the Schedules of Assessments for the SC

substudy.

A zero-time will be assigned as the start of the injection, and the residual FIX at the predose time point will be used as the baseline value for each administration. FIX activity will be measured at a central laboratory using a validated one-stage clotting method.

***Subcutaneous Substudy* Safety Variables**

Overview of Variables

Safety will be assessed based on the following variables:

- AEs and SAEs.
- investigator's assessment of local tolerability.
- subject's assessment of local tolerability.
- physical examination.
- vital signs.
- inhibitors against FIX.
- antibodies against rIX-FP.
- antibodies against CHO cell-derived proteins.
- activation of coagulation tests.

TEAEs during the subcutaneous substudy are defined as AEs with the onset of date on or after the administration of rIX-FP SC. Any AE occurring before the administration of rIX-FP SC will be considered as medical history for this subcutaneous substudy. TEAEs and SAEs will be provided for the following periods:

- during the subcutaneous period
- after SC and prior to IV in cohort 1 and 2, and after SC in Cohort 3
- after IV until the end of substudy visit for Cohort 1 and 2

Methods of Assessment

See Section 4.13 for details.

7.7 STATISTICS

Determination of Sample Size

Sample size for this SC substudy is based upon empirical considerations and no formal sample size calculation will be performed. A maximum of up to 24 subjects will be recruited into the substudy. If a subject drops out prior to Day 1, the subject may be replaced.

Analysis Populations

Please refer to Section 3.6 for definitions of analysis populations in the SC substudy.

Statistical Analyses and Methods

All safety and PK data will be summarized for the SC substudy. Continuous data will be summarized using descriptive statistics including means, SDs, medians, first and third quartiles, and minimums and maximums. Categorical variables will be summarized with frequencies and percentages.

Subcutaneous Substudy Safety Analyses

Safety data from the SC substudy will be summarized including AEs, vital signs (pulse, blood pressure, and body temperature), physical examination, investigator's and subject's assessments of local tolerability, neutralizing antibodies against FIX, antibodies against rIX-FP and CHO cells, and markers of activation of coagulation (prothrombin fragment 1+2, TAT, and D-dimer, Protocol CSL654_3001 Section 11.3.3). See Section 4.12 for details.

Pharmacokinetic Analyses

A non-compartmental analysis (NCA) will be performed on plasma FIX activity and antigen levels, if sufficient samples available, using WinNonlin® 6.2.1 (Phoenix Build 6.2.1.51) or higher. The actual sampling times will be used for PK parameter analysis. A NCA will be performed to allow individual PK data analysis.

Noncompartmental PK analysis will be performed for FIX activity in plasma, and PK parameters will be listed and summarized by treatment group. Individual subject and subgroup mean plasma concentration-time data will be plotted for each treatment group on linear and semi-logarithmic scales.

Selected NCA PK parameters will be calculated without correction for baseline FIX levels and will reflect a combination of previous IV product concentration carryover and FIX concentrations provided by subcutaneous dosing of CSL654. Baseline-corrected FIX concentrations and subcutaneous PK parameters (including %F) will be estimated using population PK methods documented in the MAP).

The following NCA PK parameters (representing total exposure resulting from IV carryover + SC FIX concentrations) will be calculated for the first dose (Cohorts 1, 2, and 3) and subsequent dose (Cohort 3) based on plasma FIX activity and antigen (if sufficient samples available) levels from rIX-FP (Table 10).

Table 10. Noncompartmental PK Parameters Representing Total Exposure (IV Carryover and SC FIX Concentrations)

IR	Incremental Recovery, defined as dose normalized activity at 30 minutes after injection ([IU/dL]/[IU/kg])
C_{max}	Maximum observed concentration/activity (IU/dL)

t_{max}	Time corresponding to C_{max} (hr)
AUC_{last}	Area under the concentration/activity-time Curve from zero (time of drug administration) to the time of last measurable (positive) concentration (t_{last}) (IU*hr/dL)
$AUC_{0-\infty}$	Area Under the concentration/activity-time Curve from zero to infinity with extrapolation of the terminal phase. (IU*hr/dL)
AUC_{ext}	Extrapolation of AUC from the last observation to infinity, expressed as a percentage of the total AUC
λ_z	First-order elimination rate constant (1/hr)
λ_z (Lower)	Lower limit on time for values to be included in the calculation of λ_z (hr)
λ_z (Upper)	Upper limit on time for values to be included in the calculation of λ_z (hr)
$t_{½}$	Terminal elimination half-life (hr)
For cohort 3 only:	
CCI [redacted]	[redacted]
CCI [redacted]	[redacted]
AUC_{0-72}	Area under the concentration/activity-time curve from zero (time of drug administration) to 72 hours after dose administration (IU*hr/dL)
CCI [redacted]	[redacted]
CCI [redacted]	[redacted]
CCI [redacted]	[redacted]

For the uncorrected concentrations, descriptive statistics (n, mean, SD, percent coefficient of variation [%CV], median, minimum, maximum, first and third quartiles (Q1, Q3) and geometric mean along with the 95% CI) will be presented. These descriptive statistics will also be presented for the uncorrected PK parameters by treatment with the exception of t_{max}, where only n, median, minimum, and maximum will be presented. PK parameters which cannot be determined will be identified in summary tables by "NC" or "NA" for not calculable or not applicable as appropriate. Descriptive statistics for PK concentration-time data and PK parameters will be stratified by dose cohort.

8 Deviations from Protocol-Stated Analyses

Deviations from the protocol-stated analyses for PTPs in Protocol CSL654_3003 (Amendment 5) are summarized below:

The analysis of safety and efficacy for subjects ≥18 years in 21-day regimen, and subjects<12 years in 14-day regimen were added to explore the prevention of bleeds in those longer prophylaxis regimens.

The endpoint of total number of exposure days of rIX-FP has been added as a secondary endpoint.

The estimate of ABRs and AsBRs (including 95% CI) are using negative binomial model as main analysis, and Poisson model as sensitivity analysis due to half of the subjects are expected to have zero bleedings (over-dispersed).

In protocol section 11.3.2.2, states that the AsBR and ABR will be derived for each regimen assigned to the subject, and will not include the time either during or the first 3 months after the surgery substudy, etc. To be consistent with pivotal studies (3001 and 3002), the AsBR and ABR will include the first 3 months after the surgery substudy.

Deviations from the protocol-stated analyses for PUPs in Protocol CSL654_3003 (Amendment 6) are summarized below:

Annualized bleeding rate for treated spontaneous bleeds and treated total bleeds will be derived and listed per subject and regimen. No comparison of AsBR or ABR between regimens will be carried out.

Deviations from SAP- stated Analyses

Any deviations from the original statistical plan will be described and justified in the final clinical study report (CSR), whether written post interim or final analysis.

9 Tables Figures, and Listings

9.1 Format and Conventions

The following conventions will be adopted for the outputs. These conventions will enhance the review process and help to standardize presentations.

- All tables, figures, and listings (where appropriate) will be presented in Landscape Orientation. A 9-*point* font size using the *Courier New* font is proposed.
- Legends will be used for all figures with more than 1 variable or item displayed.
- Any figures will be in black and white. Color figures may be produced for power-point presentations.
- Specialized text styles, such as bolding, italics, borders, shading, superscripted and subscripted text will not be used in tables and figures unless they add significant value to the table or figure.
- Only standard keyboard characters will be used in tables and figures. Special characters, such as non-printable control characters, printer specific or font specific characters, will not be used on a table or figure.

- All footnotes will be left justified and at the bottom of a page. Footnotes must be present on the page where they are first referenced. Footnotes will be used sparingly and only to add value to the table or figure. If more than four footnotes are planned than a cover page may be used to display the footnotes.
- All laboratory data will be presented in the Standard Units.
- All date values will be presented as DDMMMYYYY (e.g., 08SEP2008) format.
- All observed time values will be presented using a 24-hour clock HH:MM:SS format (e.g., 13:35:56 or 14:22). Seconds will only be reported if measured.
- Time durations will be reported in mixed HHhr MMm SSs notation (e.g., 5h 32m, or 27h 52m 20s). Decimal notations will not be used to present time durations.
- All tables and figures will have the name of the program and a date/time stamp on the bottom of each output.
- Populations represented on the tables or figures will be clearly identified in the last title of the Table.
- Consistent terminology will be used to define and identify a population.
- Population sizes will be presented for as totals in the column heading as (N=xxx), where appropriate.
- All Study population and baseline characteristics and Safety summaries will include a Total column where appropriate.
- All population summaries for continuous variables will include n, mean, SD, median, minimum, and maximum. Other descriptive statistics (e.g., quartiles, coefficient of variation) may be reported when appropriate
- The minimum and maximum values will be presented with the same number of decimal places as the raw data collected. The mean and percentiles (e.g. median, Q1, and Q3) will be presented using 1 additional decimal place. The SD and standard error will be presented using two additional decimal places. PK parameters should have a minimum of 3 significant figures.
- Percentages will be presented to 1 significant decimal place in general. The denominator will be the total size of the sample, N, unless otherwise noted.
- Percentages greater than 0 but less than 1% will be reported as <1%, whereas percentages greater than 99% but less than 100% will be reported as >99%. A percentage of 100% will be reported as 100%. No value of 0% will be reported. A computation of a percent that results in 0% will be left as blank, i.e., a numerator of 0 will be reported, however no percentage will be reported. This principle applies to portions of a whole and does not apply to differences or changes measured as percentages. Increased precision may be warranted on occasion.
- Any p-values reported on default output from statistical software may be reported at the default level of precision, with 1 exception: p-values of 0.0000 or 10E-4 or below will be reported as <0.0001.

9.2 List of Tables, Listings and Figures

The list of TLFs will be provided in a separate document that accompany the TLF shells.

10 References

Clopper C, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934; 26:404-13

Guideline on clinical investigation of recombinant and human plasma-derived factor IX products, 28 January 2016, EMA/CHMP/BPWP/144552/2009 rev 1.

SAS Institute. SAS/STAT Software: Cary, NC: SAS Institute; 2003.

Zeger SL and Liang KY. Longitudinal Data Analysis for Discrete and Continuous Outcomes. *Biometrics* 1986; 121-130.

Appendix 1

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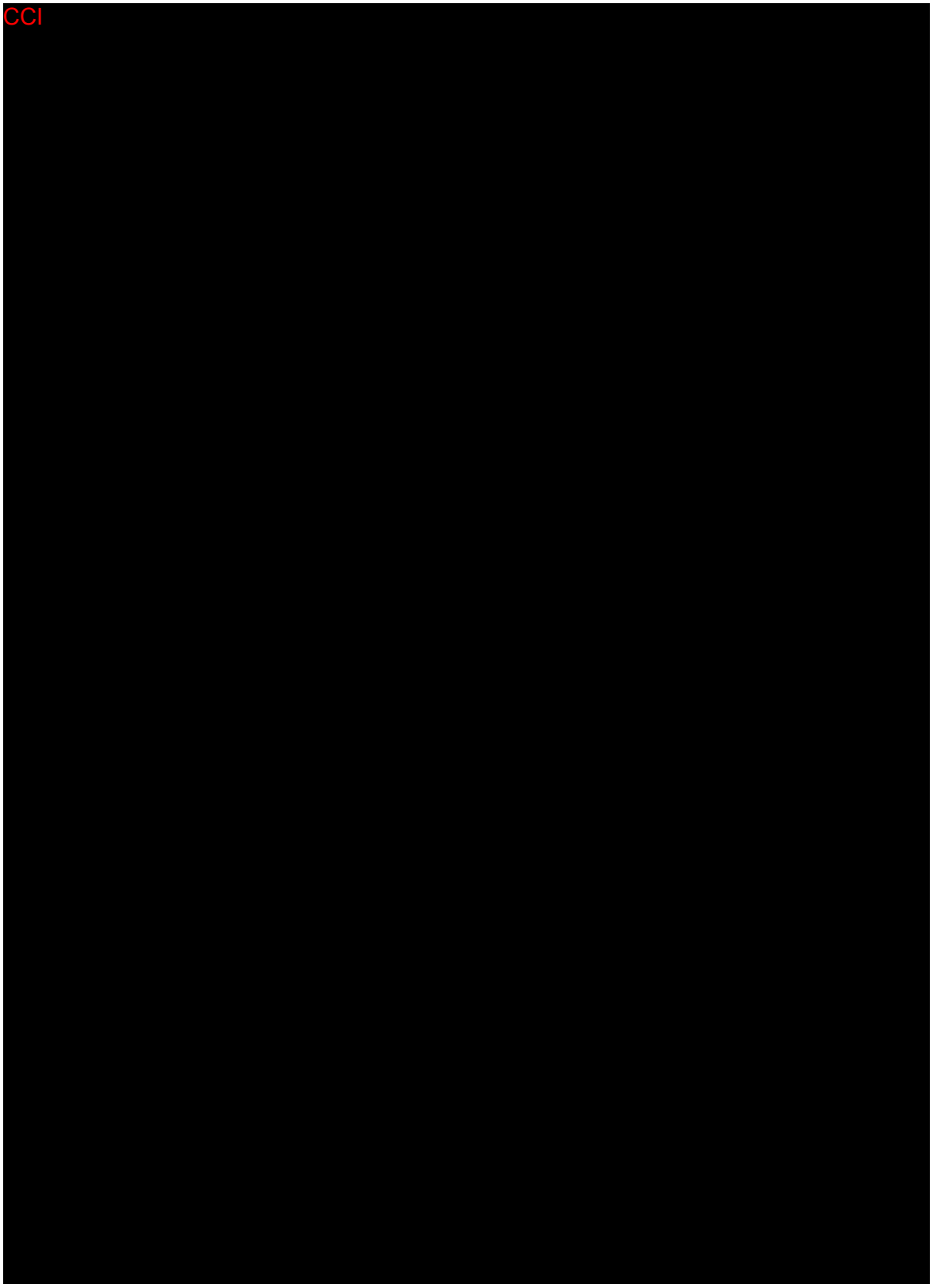

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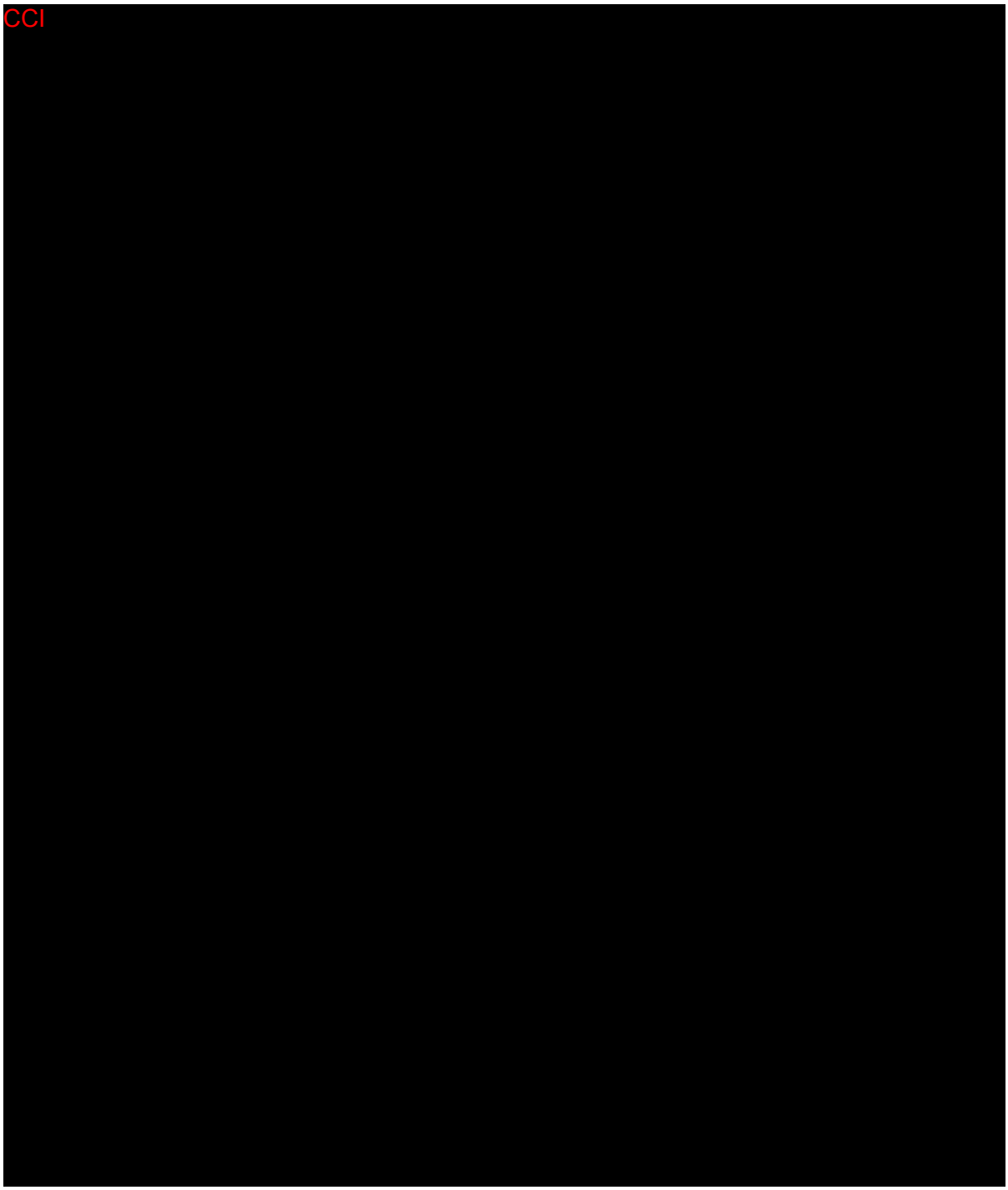
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Signature Page

CSL654_3003 - Statistical Analysis Plan - v4 - 11Dec2020

Signed By	Date (GMT)
PPD	PPD 18:07:52
Approved-PPD Approval	
PPD	PPD 17:18:28
Approved-PPD Approval	

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