Clinical Trial Protocol

Protocol No.	ISU304-001
Protocol Version/Date	5.3 (2018-09-11)
Study Drug	ISU304
Study Title	A Phase 1, Open-label, Multi-center, Dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of ISU304 in Previously Treated Hemophilia B Patients
Phase	Phase 1
Contract Research Organization	DreamCIS Inc. (CEO: Jessica Liu) 10F, Jeokseon-Hyundai B/D 130 Sajik-ro, Jongno-gu, Seoul, Korea 03170
Sponsor	ISU ABXIS (CEO: Seokju Lee) 5F, Global R&D Center Building C 22 Daewangpangyoro 712-beongil, Bundang-gu, Seongnam-si, Gyeonggi-do, Korea 13488
Study Duration	Approximately 24 months after the protocol approval from the institutional review boards (IRBs)
Confidentiality Statement	

Study Protocol Approval Page

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This protocol has been reviewed and approved by the sponsor.

Sponsor

Seokju Lee

ISU ABXIS

Signature Date

By signing below, I agree to all conditions relating to the clinical study as set out in this protocol.

I fully understand the protocol, and agree to adhere to the protocol, KGCP, and regulatory requirements in all circumstances with the only exceptions where necessary to protect the subjects.

Coordinating investigator

Churwoo You, MD

Department of Pediatrics & Adolescent Medicine, Eulji University Hospital

Signature Date

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List of Abbreviations and Definition of Terms

ADR	: Adverse Drug Reaction
AE	: Adverse Event
AESI	: Adverse Event of Special Interest
ALT (SGPT)	: Alkaline Transaminase (Serum Glutamic Pyruvic Transaminase)
aPTT	: activated Partial Thromboplastin Time
AST (SGOT)	: Aspartate Transaminase (Serum Glutamic Oxaloacetic Transaminase)
AUC	: Area Under Curve
$AUC_{(0\text{-}inf)}$	: Area Under the FIX activity time Curve from time zero to infinity
$AUC_{(0\text{-}t)}$	: Area Under the FIX activity time Curve from zero to a definite time t
BU	: Bethesda Unit
BUN	: Blood Urea Nitrogen
CL	: Total plasma clearance; the volume of blood from which the drug is total removed per unit time
C_{max}	: Observed maximum plasma concentration after administration
CRF	: Case Report Form
DMC	: Drug Monitoring Committee
DSMB	: Data Safety Monitoring Committee
FIX	: Factor IX
rFIX	: Recombinant Factor IX
GMP	: Good Manufacturing Practice
HBV	: Hepatitis B Virus
HCV	: Hepatitis C Virus
HIV	: Human Immunodeficiency Virus
IRB	: Institutional Review Board
IU	: International Unit
IV	: Intravenous
K	: Incremental recovery
KGCP	: Korean Good Clinical Practice
MedDRA	: Medical Dictionary for Regulatory Activities
MRT	: Mean Residence Time
NAb	: Neutralizing Antibody

NOAEL : No Observed Adverse Effect Level

PD : Pharmacodynamic

PK : Pharmacokinetic

PT : Prothrombin Time

RBC : Red Blood Cell

SAE : Serious Adverse Event

SC : Subcutaneous

SUSAR : Suspected Unexpected Serious Adverse Reaction

$t_{1/2}$: Terminal phase elimination half-life

TAT : Thrombin-Antithrombin Complex

T_{max} : Time to maximum plasma concentration

V_{ss} : Volume of distribution at steady state

WBC : White Blood Cell

γ-GT : Gamma-Glutamyl Transpeptidase

Synopsis

Study title	A Phase 1, Open-label, Multi-center, Dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of ISU304 in Previously Treated Hemophilia B Patients
Study centers/ Coordinating investigator	Study Centers: Eulji University Hospital Severance Hospital Pusan National University Hospital Coordinating Investigator: Prof. Churwoo You, Eulji University Hospital
Study duration	Approximately 24 months after the protocol approval from the institutional review boards (IRBs)
Indication	Previously treated hemophilia B patients
Study objective	To investigate the safety, pharmacokinetics and pharmacodynamics of ISU304 (recombinant factor IX with increased activity) in previously treated hemophilia B patients
Phase and design	Phase 1, open-label, multi-center, dose-escalation
Investigational products	• Study drug: ISU304 75, 150IU/kg, intravenous or subcutaneous administration • Active control: BeneFIX 75 IU/kg, intravenous administration • Rescue drug: Treatment currently used by subject to treat hemophilia B
Administration and follow-up period	• Cohort 1 (n=3): 7 days Single intravenous administration of BeneFIX (75 IU/kg) with 72 hours of observation, followed by single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation • Cohort 2 (n=3): 7 days Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (75 IU/kg) with 72 hours of observation • Cohort 3 (n=3): 9 days Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (150 IU/kg) with 120 hours of observation • Cohort 4 (n=5): 10 days One subcutaneous administration of ISU304 (150 IU/kg) per day for 6 days with 240 hours of observation • Cohort 5 (n=minimum 3, maximum 5): 15 days Single intravenous administration of ISU304 (75 IU/kg) is followed by

	subcutaneous ISU304 (150 IU/kg) administration for 9 days with 312 hours of observation
Planned number of subjects	At least 12 subjects
Inclusion criteria	1) Previously treated male patients with moderate or severe hemophilia B (documented FIX activity ≤ 2% and exposed to any FIX product for ≥ 150 exposure days (estimated)) 2) Patients must be 12 to 65 years old at the time of screening 3) Patients who have discontinued a previously treated FIX product at least 4 days prior to the administration of investigational product 4) HIV negative, or if HIV positive with a CD4 count > 200/μL (documented < 200 particles/μL or ≤ 400,000 copies/mL) at the time of screening 5) Voluntary consent to participate in the study
Exclusion criteria	1) Patients with a history or a family history of FIX inhibitors 2) Patients with FIX inhibitors (positive result for BeneFIX or ISU304 from inhibitor tests) at the time of screening 3) Patients who have a history of thromboembolic events (myocardial infarction, cerebrovascular disease, venous thrombosis, etc.) 4) Patients with known hypersensitivity, allergy, or anaphylaxis to any FIX product or hamster protein 5) Patients receiving treatment with a FIX product or a bypass agent within 4 half-lives for the agent used (at least 96 hours) prior to the administration of the investigational product 6) Patients who have been exposed to long-term administration (exceeding 14 days) of immunomodulating agents or immunosuppressants such as α-INF or adrenocortical hormones over the past 3 months or who are currently receiving or planning to receive such treatment during the study period 7) Patients who have been administered vaccines during the period of 6 months prior to the administration of the investigational product or plan to receive vaccines during the study period 8) Patients with any other co-existing bleeding disorder (Von Willebrand disease, etc.) 9) Patients with positive D-dimer results (≥ 0.5 μg/mL) at the time of screening 10) Patients with platelet counts less than 100,000/μL at the time of screening 11) Patients with ALT, AST levels 5 times greater than upper normal limit or total bilirubin, serum creatinine levels 2 times greater than upper normal limit at the time of screening 12) Active hepatitis patients who are HBs Ag positive or anti-HCV Ab positive and require medical treatment at the time of screening

	13) Patients scheduled for surgery during the study period 14) Patients participated in another study within 30 days before screening or scheduled to participate in any other study during the study period
Study timeline/ Data collection	This study is a phase 1, open-label, multi-center, dose-escalation study to investigate the safety, pharmacokinetics and pharmacodynamics of ISU304 in previously treated hemophilia B patients. All subjects shall give their written consent before participating in this study and only those who meet all of the inclusion criteria and none of the exclusion criteria will be administered the investigational products. This study is comprised of 5 cohorts. Each cohort will receive an identical intravenous administration of 75 IU/kg, with subcutaneous administrations doubling from 75 IU/kg until 150 IU/kg. During the study period, a subject may be hospitalized to facilitate the collection of blood samples for pharmacokinetic (PK)/pharmacodynamic (PD) analysis. Hospitalization itself will not be considered as a serious adverse event if no adverse event occurs. PK/PD analysis and safety assessments will be conducted for each cohort. The Data Safety Monitoring Board (DSMB) and Drug Monitoring Committee (DMC) will be operated after the end of Cohorts 1 to 4. These committees will monitor the PK/PD and safety data from each cohort to determine the continuation of next cohort (Cohorts 2 to 5), target dose, and blood sampling period for PK/PD (including timing of collection). Additional subjects may be enrolled in all cohorts or cohorts may be canceled depending on the results of PK/PD analysis.
Safety endpoints	• Adverse events after the administration of investigational products (local/systemic/other)[1] • Physical examination[2] • Vital signs • Electrocardiogram • Laboratory tests • FIX inhibitors (or neutralizing antibodies) and anti-drug antibodies to investigational products
Pharmacokinetic/ Pharmacodynamic endpoints	• PK endpoints: observed maximum plasma concentration (C_{max}), terminal phase elimination half-life ($t_{1/2}$), total plasma clearance (CL), volume of distribution at steady state (V_{ss}), area under curve (AUC; $AUC_{(0-t)}$ and $AUC_{(0-inf)}$), mean residence time (MRT), incremental recovery (K), time to maximum plasma concentration (T_{max}) for FIX activity and FIX product • PD endpoint: activated partial thromboplastin time (aPTT)
Other endpoints	Demographic data, hemophilia B status, medical history, medication history

[1] Including adverse events of special interest (AESI)
[2] Includes assessment of tolerability of subcutaneous injection (excluding Cohort 1), local reactions in administration site and systemic reactions after subcutaneous administration of investigational products.

Analysis method	• Analysis population - Safety analysis set: All subjects who receive at least one dose of investigational products - PK set: Subjects in the safety analysis set who complete all procedures planned for the cohort and for whom PK samples[3] have been obtained at least 4 times after administration. • PK Analysis: Using WinNonlin software (Pharsight, CA, USA)[4] • Bioavailability analysis for subcutaneous administration • Other statistical analysis - For each cohort, descriptive statistics will be given for each endpoint for each subject using SAS software (SAS Institute Inc, Cary, USA)

[3] The 4 PK sampling points should include at least 2 points within 3 hours after IV administration, or within 12 hours after SC administration. However, Cohort 4 and Cohort 5 subjects can be included in the PK set with samples from 6 hours after the 1st administration and 6 hours after the 6th administration.

[4] NONMEM software (ICON, MD, USA) will be used for additional analysis, if necessary.

Study Schedules and Procedures

Visit no.	Screening	1st/2nd Hospitalization	Administration of investigational product and timing of blood collection for PK/PD analysis[a]														Interim visit /End of a cohort	Post-study safety visit
1st administration period	-30 ~ 0	-1 ~ 0	0							1	2	3	4	5	6			
2nd administration period		4 ~ 5	5							6	7	8	9	10	11		3/5(or 6)	+14 (+7)
Hours after administration	**NA**	**NA**	**Before administration**	**0**					**6**		**24**	**48**	**72**	**96**	**120**	**144**	**NA**	**NA**
Informed consent form	X																	
Demographics[b]	X																	
Hemophilia B status[c]	X	X																
Medical and medication history[d]	X	X																
Physical examination	X		X						X		X	X	X	X†	X	X†	X	
Vital signs[e]	X		X						X		X	X	X	X†	X	X†	X	
Electrocardiogram[f, *]	X	X															X	
Inclusion/exclusion criteria	X	X[1)]																
Administration of investigational product				X														
Laboratory tests (central laboratory)[g, *]																		
Hematology	X	X															X	
Blood chemistry	X	X															X	
Serology	X																	
Urinalysis	X	X															X	
Blood coagulation	X[2)]	X							X		X	X	X	X†	X	X†	X	
BeneFIX anti-drug antibody/ neutralizing antibody	X	X[3)]															X	X
ISU304 anti-drug antibody/ neutralizing antibody	X	X															X	X
Bethesda assay		X															X	X
Safety assessment[h]																		
Concomitant medications		X[4)]	X[4)]	X														X
Adverse events				X[5)]														X

Study Schedules and Procedures (Cont'd)

		Screening	1st/2nd Hospitalization	Administration of investigational product and timing of blood collection for PK/PD analysis[a]														Interim visit /End of a cohort	Post-study safety visit
Visit no.	1st administration period	-30 ~ 0	-1 ~ 0	0								1	2	3	4	5	6		
	2nd administration period		4 ~ 5	5								6	7	8	9	10	11		+14 (+7)
Hours after administration(±min)	Intravenous administration	N/A	N/A	Before administration (-30)	0 (+1)	0.25 (±3)	0.5 (±3)	1 (±3)	3 (±5)	6 (±5)	9 (±5)	24 (±15)	48 (±120)	72 (±120)	96 (NA)	120 (±180)	144 (NA)	NA	NA
	Subcutaneous administration				1 (+3)	2 (±3)	4 (±5)	6 (±5)	8 (±5)	10 (±5)	12 (±5)								
PK/PD analysis (blood collection)[i]																			
Cohort 1	BeneFIX 75 IU/kg w/IV	-	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-	-
	ISU304 75 IU/kg w/IV	-	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-	-
Cohort 2	ISU304 75 IU/kg w/IV	-	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-	-
	ISU304 75 IU/kg w/SC	-	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-	-
Cohort 3	ISU304 75 IU/kg w/IV	-	-	X	X	X	X	X	X	X	X	X	X	X	-	-	-	-	-
	ISU304 150 IU/kg w/SC	-	-	X	X	X	X	X	X	X	X	X	X	X	-	X	-	-	-
Cohort 4[j]	ISU304 150 IU/kg w/SC	-	-	X‡	-	-	-	X‡	-	-	-	X‡	-	-	-	-	-	-	-
Cohort 5[k]	ISU304 75 IU/kg w/IV	-	-	X	X	-	X	-	-	-	-	-	-	-	-	-	-	-	-
	ISU304 150 IU/kg w/SC	-	-	X♀	-	-	-	-	-	-	-	X♀	-	X♀	-	X♀	-	-	-
Hospitalization/discharge[k]																			
Hospitalization			X																
Discharge												X							

w/ - with; IV - intravenous administration, SC - subcutaneous administration

*For Cohort 4 and Cohort 5, there are no hospitalizations or interim visits, thus replaced by date of 1st administration of the investigational product.

†- Only for Cohort 4 and Cohort 5

‡- Blood collection for PK/PD analysis in Cohort 4 take place right before each administration, 6 hours after 1st administration, 6 hours after 6th administration, and 24 hours after 6th

administration.

♀- Blood collection for PK/PD analysis in Cohort 5 take place before intravenous administration, after administration, 30 min and right before subcutaneous administration, and 24, 72 and 120 hours after 9th subcutaneous administration.

a. Additional visit beyond the designated will be conducted upon request from a subject or subject's representative, or by the investigator's decision. Additional subjects may be enrolled in all cohorts or cohorts may be canceled depending on the results of PK/PD analysis.

b. Gender, date of birth, age, weight, and height

c. Documentation of the lowest FIX activity, year of diagnosis, family history of hemophilia B, history and family history of FIX inhibitors, and previous FIX treatment history

d. Subject's past (within 3 months prior to screening) or current medical history and medication history (within 3 months prior to screening, within 6 months for vaccines)

e. Blood pressure, pulse rate, respiration rate, and body temperature

f. Actual recording date/time, PR, QRS, QT, corrected QT intervals, and investigator's assessment

g. All hematology, blood chemistry, blood coagulation, serology, urinalysis, and Bethesda assay shall be conducted at the central laboratory (SCL; Seoul Clinical Laboratories, KR). Anti-drug antibody/neutralizing antibody tests for BeneFIX and ISU304 shall be conducted at the other central laboratory (HTI, Haematologic Technoilgies Inc., USA). However, tests requiring analysis within 24 hours (hematology, urine test) can be conducted at each site. As aPTT is a PD endpoint, it is collected during the PK/PD sampling period. Sample storage and disposal will be carried out according to central laboratory's recommended procedure. For laboratory test conducted for confirmation of inclusion/exclusion criteria, retest is allowed during the screening period as judged by the Investigator.

Hematology: RBC, WBC with differential count (Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil), Hemoglobin, Hematocrit, Platelet count, CD4 count (Screening only)

Blood chemistry: Total protein, Albumin, Creatinine, BUN, Uric acid, Total bilirubin, AST (SGOT), ALT (SGPT), γ-GT

Blood coagulation: aPTT, PT international normalized ratio, PT sec, TAT, D-dimer, F1+2, Fibrinogen

Serology: anti-HBV (HBs Ag), anti-HCV (Anti-HCV Ab), anti-HIV (Anti-HIV Ab)

Urinalysis: pH, Protein, Glucose, Urobilinogen, Microscopic exam (if abnormal dipstick test): RBC, WBC

h. Safety endpoints are collected until the end of each cohort.

i. PK/PD analysis are carried out at the designated central laboratory (HTI). Target dose, and blood sampling period (including timing of collection) in Cohorts 2 to 4 will be determined based on the PK/PD analysis of the preceding cohort. Subjects from Cohort 1 ~ 3 are expected to participate in Cohort 4.

The following endpoints will be estimated and analyzed at the central laboratory (HTI) for each subject: observed maximum plasma concentration (C_{max}), terminal phase elimination half-life ($t_{1/2}$), total plasma clearance (CL), volume of distribution at steady state (V_{ss}), area under curve (AUC; $AUC_{(0-t)}$ and $AUC_{(0-inf)}$), mean residence time (MRT), incremental recovery (K), time to maximum plasma concentration (T_{max}) for FIX activity and FIX product

j. The investigational product is administered daily for 6 days.

k. ISU304 75 IU/kg is intravenously administered at the first day and this is followed by subcutaneous administration of ISU304 150 IU/kg within 30 min. Afterwards, ISU304 150 IU/kg is subcutaneously administered once a day for 8 days, with a 24 hour interval.

l. Subjects who have completed blood collection during hospitalization may make outpatient visits for the remaining blood sample collections. Cohort 4 does not need hospitalization.

1) The final decision on eligibility is made based on data collected during screening.

2) Only D-dimer is assessed at screening.

3) Subjects in Cohort 1 who have been administered BeneFIX will be tested for BeneFIX anti-drug antibodies and neutralizing antibodies.

4) Information on previous concomitant medication may be collected before the 2nd administration of the investigational product within the same cohort.

5) AEs including injection site reactions must be observed for at least 30 minutes to 1 hour after the administration of the investigational product.

1 Study Background and Objective

1.1 Background

Hemophilia B is an X chromosome related congenital bleeding disorder caused by a deficiency of the clotting factor IX (FIX). This disorder is caused by a mutation of the clotting factor, and it typically affects males while females are usually carriers(1). The worldwide prevalence of hemophilia B ranges from 0.9 to 3.2 cases per 100,000 males(2).

FIX activity is usually expressed in international units (IU/mL), wherein 1 IU/mL (100 IU/dL) represents 100% FIX activity in 1 mL of normal blood plasma. The FIX activity range of normal individuals is about 0.5 ~ 1.5 IU/mL (50 ~ 150% or 50 ~ 150 IU/dL), and varying symptoms of hemophilia occur at lower activity levels(3). Patients whose FIX activity is below 0.01 IU/mL (< 1%) are classified as severe cases, who experience spontaneous bleeding in the joints and muscles. In some cases, bleeding can be life-threatening and require immediate treatment. While the symptoms of bleeding are usually life-long, some children with severe hemophilia may not have bleeding symptoms until later when they begin walking or running. Patients with mild hemophilia may not bleed excessively until they experience trauma or surgery(1).

Bleeding can be prevented and hemostasis can be induced when bleeding occurs in severe hemophilia patients by supplementing the deficient clotting factor. In other words, FIX products can be administered as prophylaxis therapy (administration of clotting factor to prevent spontaneous or trauma induced bleeding) or as on-demand therapy to induce hemostasis when bleeding occurs (administered in the presence of clinically clear bleeding). Prophylaxis is a form of therapy that prevents bleeding and joint damage, with the goal of preserving normal musculo-skeletal functions(1).

Plasma-derived FIX products have made prophylaxis therapy possible since 1970s. Due to safety issues, however, recombinant FIX (rFIX) products have been used more frequently in the treatment of hemophilia B in recent decades(4). The first-generation rFIX products have relatively short half-lives, which necessitate frequent infusion. To address this issue, the second-generation products combined with substances that can increase the half-life of FIX are being developed. The second-generation rFIX products have been used for prophylaxis therapies by intravenous administration once a week to once every two weeks. Although the second-generation products have reduced the inconvenience of frequent infusions associated with the first-generation products, there is still room for improvement as these products still have to be administered intravenously. rFIX products currently approved for the Korean market include BeneFIX (Pfizer Ltd.) and Rixubis (Baxalta Inc.) but the second-generation rFIX products are still yet to be approved.

1.2 Theoretical Rationale

All FIX products currently available are administered intravenously. While intravenous administration is useful for hemostasis during acute bleeding, it is inconvenient for patients who require long-term prophylaxis therapy due to frequent administration schedules.

Subcutaneous administration is generally preferred over intravenous administration because it enables at-home injection, improves the quality of life, and reduces health care costs[6]. Although biological agents such as insulin, growth hormones and antibodies have been developed as subcutaneously administered products, a subcutaneous FIX product has yet to be developed. Due to the characteristics of the administration route, subcutaneous products are limited in terms of administration volume (typically 1 ~ 2 mL). Currently developed FIX products have low activity levels per unit volume (maximum of 600 IU/mL for BeneFIX), making them unsuitable for subcutaneous administration with volume limitations. For example, a 50 IU/kg dose to a patient who weighs 60 kg, would require a total dose of 3,000 IU, which translates to 5 mL in administration volume. The low bioavailability of subcutaneous FIX injections also makes subcutaneous injections more challenging. In nonclinical studies on animal subjects, subcutaneous bioavailability of FIX ranged from 25 to 86% according to species[7, 8, 9]. Liles et al (1997) proposed a subcutaneous bioavailability of 33% from one hemophilia B patient[9]. The low bioavailability means that subcutaneous administration requires higher doses compared to intravenous and thus the volume also increases. With the increase in administration volume, treatment costs are also expected to escalate.

In order to make a FIX product that can be injected subcutaneously, a FIX product with a high level of specific activity had to be developed. With high specific activity, the FIX activity per unit volume also increases, reducing the administration volume required. As the amount of FIX required also decreases with high specific activity, there is the added advantage of being able to produce highly concentrated FIX products without a corresponding increase in production cost.

In order to produce an rFIX product with high coagulation activity, a structure-based rationale design was employed. Through this method, we produced a wide range of FIX variants, to develop a modified FIX (named ISU304) with high coagulation activity through biological screening (Patent No.: WO2012061654 A1). ISU304 was created by introducing 3 point mutations in 2 loops within the FIX protein. With these point mutations, arginine-318 is transformed into tyrosine (Arg318Tyr), arginine-338 into glutamic acid (Arg338Glu), and threonine-343 into arginine (Thr343Arg). One of the mutations (Arg318Tyr) is located in ‘loop-150’, which is also known as the autolysis loop, while the other two (Arg338Glu and Thr343Arg) are located in ‘loop-170’. The mutation in ‘loop-170’ can significantly enhance affinity to the cofactor VIII and stabilize activated FIX (FIXa). The mutation in

'loop-150' is also expected to stabilize the activation structure of FIXa, as well as directly interact with factor X, a substrate of FIX and Anti-thrombin III (ATIII), a key inhibitor. In conclusion, these minimal mutations in the 3D molecular structure (3 amino acid mutations) allow ISU304 to demonstrate 3 times the catalytic efficiency to factor X (substrate), 10 times higher affinity to cofactor FVIIIa and 15 times the resistivity to inhibitor ATIII compared to wild-type FIX. These characteristics resulted in ISU304 demonstrating 20 times the in vitro coagulation activity as well as 20 times increased titer in hemostatic potency study with hemophilic mice models compared to BeneFIX.

The pharmacokinetic characteristics of ISU304 in subcutaneous and intravenous administrations have been observed in animal tests. ISU304 and BeneFIX displayed similar pharmacokinetic profiles when identical doses of each (75 IU/kg and 250 IU/kg) were intravenously administered to hemophilic mice. Subcutaneously administered ISU304 and BeneFIX demonstrated respective bioavailabilities of 16 ~ 22% and 30 ~ 35% in normal mice and minipig with half-lives increasing two-fold or more in subcutaneous administration compared to intravenous administration. When ISU304 was subcutaneously administered daily in 230 IU/kg doses to hemophilic mice with severe hemophilia FIX activity levels (< 1.1 IU/dL), they were able to maintain FIX activity as mild hemophilia levels (> 5 IU/dL). When 300 IU/kg was subcutaneously administered daily to hemophilic dogs for 6 days, FIX activity in plasma increased up to 50 IU/dL, which falls within the FIX activity range of normal individuals (50 ~ 150 IU/dL). With the increase in plasma FIX activity, a decrease in whole blood coagulation time was also observed.

In conclusion, ISU304 is a FIX product that shows markedly improved specific activity compared to existing products. Currently developed ISU304 product offers per unit volume activity levels (> 7,500 IU/mL) over 10 times higher than the maximum activity of BeneFIX (600 IU/mL), making it possible to administer 75 ~ 300 IU/kg doses to patients that weigh 70 kg, with dose volumes of just 0.7 ~ 3 mL. It was also demonstrated that coagulation activity levels akin to mild hemophilia patients or normal individuals could be maintained through repeated subcutaneous administrations. Therefore, ISU304 is deemed to be an appropriate product for subcutaneous injection as originally aimed and the objective of this clinical study is to verify this estimation.

1.2.1 Results from Toxicity Studies

Toxicity studies on ISU304 consistently showed that ISU304 was well tolerated in mice, rats, monkeys, and rabbits. All of them showed there was no death or clinically significant findings caused by the study treatment. The maximum tolerated doses in rats and monkeys were above 3.0 mg/kg (12,900 ~ 15,200 IU/kg) when intravenously administered 2 times a week for 4 weeks, and above 1.0

mg/kg (4,600 IU/kg) for daily subcutaneous administrations over 2 weeks in rats. The table below summarizes the preclinical toxicity studies.

Type of study	Animal species/strain	Administration method	Administration period	Dose (mg/kg[a])	Study No.
Preliminary toxicity	Mouse/ICR	IV	1 day	0, 0.3, 1, 3, 10	15-KE-056
Acute toxicity	Rat/CD	IV	2 weeks	0, 0.3, 3	2470-007
	Monkey/ Cynomolgus	IV	2 weeks	0, 0.3, 1, 3	2470-008
Repeated administration toxicity, local tolerance, antigenicity	Rat/CD	IV	4 weeks	0, 0.3, 1, 3	2470-002
	Monkey/ Cynomolgus	IV	4 weeks	0, 0.3, 1, 3	2470-003
	Rat/CD	SC	2 weeks	0, 0.1, 0.3, 1	2470-012
Other toxicity					
Plasma generation	Rabbit/NZW	IV	10 min	0, 0.3, 1, 3	331676

†- Underline indicates no-observed-adverse-effect level (NOAEL)

Specifically, study15-KE-056 investigated the toxicity of a single intravenous administration of ISU304 in mice. There were no deaths or clinically significant findings caused by study treatment. The results indicated that the maximum tolerated dose was above 10 mg/kg (54,000 IU/kg) for a single intravenous dose of ISU304 administered to mice.

Studies 2470-007 and 2470-008 estimated maximum tolerated doses in rats and monkeys respectively, by administrating repeated weekly intravenous doses of ISU304 over two weeks. For both studies, there were no deaths or clinically significant findings caused by study treatment. The results indicated that the maximum tolerated doses were above 3.0 mg/kg (15,100 IU/kg) for repeated intravenous administrations of ISU304 to rats and monkeys.

Additionally, studies 2470-002 and 2470-003 were repeated dose toxicity studies with intravenous doses of ISU304 administered twice a week over 4 weeks to rats and monkeys respectively. For both studies, there were no deaths or clinically significant findings caused by study treatment. The results indicated that the maximum tolerated doses were above 3.0 mg/kg (12,900 ~ 15,200 IU/kg) for repeated intravenous administrations of ISU304 to rats and monkeys.

Study 2470-012 administered ISU304 daily to rats for over 2 weeks to test the toxicity of subcutaneous administration. There were no deaths or clinically significant findings caused by study

treatment. The results indicated that the maximum tolerated dose was above 1.0 mg/kg (4,600 IU/kg) for repeated intravenous administration of ISU304 to rats and monkeys.

Study 31676 examined the thrombogenic risk in rabbits after intravenous administration of ISU304 or BeneFIX. Wessler's stasis model showed that there was no thrombosis across dose levels until 3.0 mg/kg. ISU304 had shorter activated partial thromboplastin time (aPTT) and longer prothrombin time (PT) compared to BeneFIX. The no-observed-adverse-effect-level (NOAEL) was defined as 3.0 mg/kg (15,100 IU/kg).

The above results clearly demonstrate the safety of ISU304. The maximum intravenous dose for ISU304 in this study has been defined as 75 IU/kg (0.0335 mg/kg), 260 times lower than the maximum tolerated dose of 12,900 ~ 15,200 IU/kg (3 mg/kg) verified in the pre-clinical studies. The maximum subcutaneous dose for ISU304 is 300 IU/kg, about 12 times lower than the maximum tolerated dose of 4,600 IU/kg (1 mg/kg) verified in the pre-clinical studies. Therefore, the ISU304 administration doses to be used in this clinical study are deemed to be well within the range of safety based on the results of preclinical toxicity studies.

1.2.2 Results from Pharmacokinetic/Pharmacodynamic Studies

The pharmacokinetic/pharmacodynamic characteristics of ISU304 were examined in previous studies. The table below summarizes the outcomes of these preclinical pharmacokinetic/pharmacodynamic studies.

Type of study	Animal species	Administration method	Dose (mg/kg)	Results	Study No.
Blood concentration after single dose	Hemophilic mice	IV	· ISU304: 0.05, 0.19, 0.75, 3.0 (250, 1,000, 4,000, 16,000 IU/kg) · BeneFIX: 0.92 (250 IU/kg)	- Proportionate increases in C_{max} and AUC to increases in ISU304 dose. - Similar pharmacokinetic parameters to antigens ($t_{1/2}$, C_{max}, C_0, AUC_{0-t}, AUC_{0-inf}, MRT) when ISU304 and BeneFIX with similar amounts of protein are administered.	15-KE-190
	Hemophilic mice	IV	· ISU304: 0.01 (75 IU/kg) · BeneFIX: 0.28 (75 IU/kg)	- Similar pharmacokinetic profiles (C_{max}, MRT, $t_{1/2}$) and systemic exposure (< 2 fold difference) to ISU304 and BeneFIX activity when identical activated doses (75 IU/dL) are administered. - The high specific activity of ISU304 causes significantly lower exposure in terms of antigens compared to BeneFIX.	17-KE-036

Type of study	Animal species	Administration method	Dose (mg/kg)	Results	Study No.
	Normal mice	SC IV	· ISU304, SC: 0.02, 0.05, 0.15 (90, 230, 690 IU/kg) · ISU304, IV: 0.05 (230 IU/kg) · BeneFIX, SC: 0.15 (42 IU/kg)	- Increased half-life of ISU304, decreased C_{max} in subcutaneous administration compared to intravenous. - BA of ISU304 (17 ~ 22%) and BeneFIX (16%) are similar.	16-KE-091
	Monkeys	IV	· ISU304, IV: 0, 0.3, 1.0, 3.0 (0, 1,510, 5,032, 15,096 IU/kg)	- C_{max} and AUC increased along with an increase in intravenous ISU304 dose to monkeys. - Pharmacokinetic parameter comparison between groups difficult, due to quantitative restrictions.	2470-009
	Rats	IV	· ISU304, IV: 0, 0.3, 1.0, 3.0 (1,510, 5,032, 15,096 IU/kg)	- C_{max} and AUC_{0-t} increased in proportion to the intravenously administered ISU304 dose. - Half-life of ISU304 in rats is 22 ~ 26 h.	2470-010
	Hemophilic mice	SC	· ISU304, SC: 0.02, 0.05, 0.15 (90, 230, 690 IU/kg)	- When ISU304 is subcutaneously injected in escalating doses, the plasma concentration and activity of ISU304 increased in proportion. - The in-plasma levels of ISU304 antigen and activity measured after subcutaneous administration are linearly proportional to each other.	16-KE-091
	Minipigs	SC IV	· ISU304 SC: 0.05, 0.15 (230, 690 IU/kg) · ISU304 IV (230 IU/kg) · BeneFIX SC: 0.15 (42 IU/kg)	- Similar pharmacokinetic profiles between subcutaneously administered ISU304 and BeneFIX. - Bioavailability of subcutaneously administered ISU304 and BeneFIX is 30 ~ 35%. - C_{max} is 6.6 ~ 10.8% lower in subcutaneous administration compared to intravenous administration. - Half-life of ISU304 (27 ~ 30 h) is longer than dose interval (24 h) in subcutaneous administration, leading to accumulation in plasma when administered repeatedly.	16-KE-135
	Hemophilic dogs	SC IV	· ISU304, IV: 50 IU/kg · ISU304, SC: 42, 72, 125, 143 IU/kg	- Bioavailability of subcutaneously administered ISU304 is approximately 14%. - Bioavailability of subcutaneously administered ISU304 and BeneFIX is 30 ~ 35%. - C_{max} is 6.6 ~ 10.8% lower in subcutaneous administration compared to intravenous administration.	Internally managed

Type of study	Animal species	Administration method	Dose (mg/kg)	Results	Study No.
Blood concentration after repeated doses	Hemophilic mice	IV	· ISU304: 0.94 (5,000 IU/kg) · 1 dose/week for 3 weeks	- When ISU304 was administered once a week for 3 weeks in 5,000 IU/kg (0.92 mg/kg) doses, accumulation was not observed. - Plasma antigen and activity levels of ISU304 are linearly proportional to each other.	15-KE-194
	Hemophilic mice	SC	· ISU, SC: 0.05 (230 IU/kg) · Once daily for 4 days	- Insignificant tendency towards accumulation when ISU304 is subcutaneously administered every day.	16-KE-091
	Minipigs	SC	· ISU304, SC 0.1 (460 IU/kg) · Once daily for 6 days	- Half-life of ISU304 (27 ~ 30 h) is longer than dose interval (24 h) in subcutaneous administration, leading to accumulation in plasma when administered repeatedly.	16-KE-135
	Hemophilic dogs	SC	· ISU304, SC 300 IU/kg · Once daily for 6 days	- Half-life of ISU304 (> 50 h) is longer than dose interval (24 h) in subcutaneous administration, leading to accumulation in plasma when administered in repeated daily doses.	Internally managed

* AUC: area under curve, IV: intravenous, SC: subcutaneous

In study 15-KE-190, mice with FIX deficiency (hemophilic mice) were administered with single doses of ISU304, BeneFIX, or Alprolix. The results indicated that C_{max} and area under curve (AUC) proportionally increased as administered dose increased, but half-life was relatively consistent across dose levels, with a range of 19.3 to 23.4 hours. ISU304 and BeneFIX had similar half-lives while the half-life of Alprolix was about double the length of ISU304 and BeneFIX. Identical intravenous active doses (250 IU/kg) of ISU304 and BeneFIX showed similar pharmacokinetic profiles (AUC, $t_{1/2}$, MRT, Cl, AUMC, Vz).

Study 16-KE-036 administered single intravenous ISU304 and BeneFIX doses to hemophilic mice according to the dose to be administered in the clinical study (75 IU/kg) and analyzed their pharmacokinetic characteristics. The two drugs showed similar pharmacokinetic profiles in terms of FIX activity (C_{max}, AUC, MRT, $t_{1/2}$). The C_{max} and AUC of FIX antigen differed between the two substances due to the difference in administered antigen volume, but the half-lives and MRT were similar. Study 15-KE-194 administered ISU304 in 3 repeated intravenous weekly doses to FIX deficient mice. The results showed that C_{max}, AUC, and half-life were consistent across periods, which meant ISU304 was not accumulated in mice.

Studies 2470-009 and 2470-010 investigated pharmacokinetics of FIX following a single intravenous administration of ISU304 in monkeys and rats, respectively. The results showed pharmacokinetic characteristics across species.

The recent studies of 16-KE-091 and 16-KE-135 analyzed pharmacokinetic characteristics of subcutaneously administered ISU304 in mice. While the subcutaneous administration of BeneFIX showed 16% bioavailability in mice and 30% bioavailability in minipigs, the subcutaneous administration of ISU304 showed 19 ~ 22% bioavailability in mice and 30 ~ 35% bioavailability in minipigs. When identical doses of ISU304 were administered to minipigs subcutaneously and intravenously, C_{max} was approximately 7 ~ 11% lower in subcutaneous administration compared to intravenous administration but half-life was about 2.6 times longer. When 690 IU/kg (0.15 mg/kg) of ISU304 was subcutaneously administered to minipigs, C_{max} was 141 ng/mL, which translates to 49% (49 IU/dL) in terms of activity. Supposing that subcutaneously injected ISU304 has similar C_{max} in humans and minipigs, a single dose up to 690 IU/kg is expected to produce a FIX activity level below the range of normal individuals (50 ~ 150%).

1.2.3 Rationale for Dose Determination

This phase 1 clinical study has been planned to investigate the safety, pharmacokinetics and pharmacodynamics of intravenously and subcutaneously administered ISU304 in previously treated hemophilia B patients. The initial dose and dose escalation plan has been determined according to the results of the toxicity studies after intravenous administration in rats and monkeys, the toxicity studies after subcutaneous administration in rats, the pharmacokinetic tests after subcutaneous and intravenous administration of ISU304 and BeneFIX in hemophilic mice and minipigs, as well as the results of pharmacokinetic studies after intravenous and subcutaneous administrations of FIX as reported in the existing literature.

1) Single intravenous administration

The initial intravenous dose of ISU304 has been set as 75 IU/kg(approximately 0.015 mg/kg) while the post-administration blood collection period has been set as 3 days (72 hours). The initial dose of 75 IU/kg for ISU304 is identical to the clinical study dose of BeneFIX, a currently marketed hemophilia B treatment product. The blood sample collection period for PK measurements is also identical to that in the same study (Lambert et al., 2007). The dose is also approximately 200 times lower than the NOAEL identified in the preclinical toxicity study for intravenous administration (3 mg/kg/dose (approximately 13,000 ~ 15,000 IU/kg)).

In the result of a pharmacokinetic study, that was carried out after intravenous administration of the same dose (75 IU/kg and 250 IU/kg) of ISU304 and BeneFIX in hemophilic mice, similar pharmacokinetic profiles between the two substances were confirmed (Fig. 1.1 (A)).

In the EMA approval assessment report for Rixubis (EMEA/H/C/003771/0000), a biosimilar product of BeneFIX, the result of comparative PK in hemophilic mice and monkeys in the non-clinical study showed a similarity in their PK profiles. The similar PK profiles between two products were also confirmed in humans through comparative PK clinical studies.

Considering the results of the above tests and non-clinical/clinical studies of similar products, it is deemed that similar PK profiles in animal testing predict similar PK profiles in humans as well. Therefore, since ISU304 and BeneFIX show similar PK profiles in animals, it may be predicted that they will show similar PK profiles in humans as well. Consolidating all results, it is expected that the intravenous administration of ISU304 (75 IU/kg) will show a PK profile similar to BeneFIX. This dose concentration is deemed to be safe as it falls within the approved range for BeneFIX (50 ~ 100 IU/kg) (Product Monograph for BeneFIX, 2012) and the normal range for humans (50 ~ 150 IU/dL).

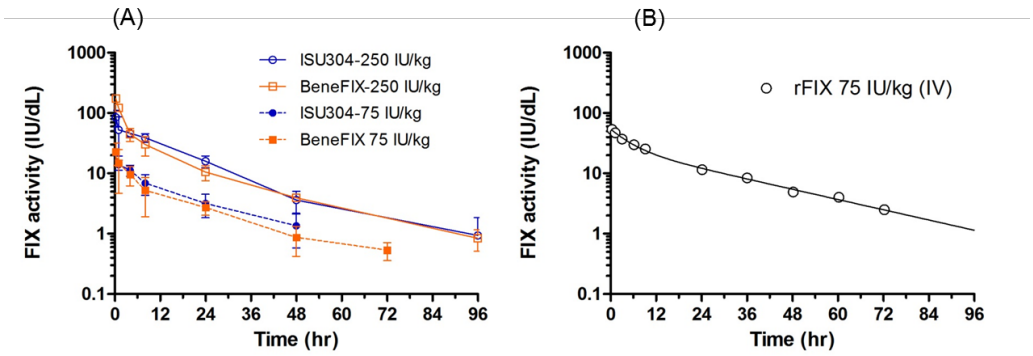


Fig 1.1 The time-concentration curves of FIX in hemophilic mice and hemophilia patients (A) The comparison of PK between ISU304 and BeneFIX in hemophilic mice, (B) The plasma concentration-time profile of 75 IU/kg rFIX (BeneFIX) intravenously administered to hemophilia patients (Lambert et al., 2009)

According to the clinical study data for BeneFIX reported in the above literature (Fig. 1.1 (B)), when 75 IU/kg of FIX is intravenously administered, C_{max} is approximately 55 IU/dL while $AUC_{0\text{-}72h}$ is 851±215 IU/dL*h, and blood concentration falls to 2.5 IU/dL 72 hours after administration (Fig 1.1(B): Lambert et al., 2009).

Blood concentration is expected to drop to 1 ~ 2%, 72 ~ 96 hours after a 75 IU/kg dose of ISU304 in this clinical study as well, similarly to BeneFIX. Thus, the blood collection period of 72 hours is deemed to be appropriate considering the subject's safety (Product Monograph for BeneFIX, 2012).

2) Single subcutaneous administration

As there is no commercially available rFIX protein product developed for subcutaneous administration, the initial subcutaneous doses in this clinical study have been set as 75, 150, and 300 IU/kg, based on animal testing results (on normal mice, hemophilic mice, hemophilic dogs and minipigs, etc.) obtained in the course of the development of ISU304, as well as results reported from a study on subcutaneous administration of FIX on humans.

The aspects of NOAEL, AUC and C_{max} have been considered in the initial doses and adminis tration periods planned in this study, andthe rationale for determination of the initial subcutan eous injection dose could be obtained as shown in .

Table 1.1.

Table 1.1 The rationale for Determination of Initial Subcutaneous Injection Dose

Administration Route	Data	Species	NOAEL			AUC		C_{max}	
			Dose		Relative value	IU/dL*h	Relative value	IU/dL	Relative value
			mg/kg	IU/kg					
IV Injection	Toxicokinetics	Monkeys	3	14,826	198	1,164,928	1,369	56,882	1,034
		Rats	3	12,915	172	129,581	152	20,061	364
	Clinical Design[1)]	Humans	0.01	75	1	851	1.0	55	1.0
SC Injection	Toxicokinetics	Rats	1	4,305	57	933[2)]	1.5	49	0.9
	Clinical Design[3)]	Humans	0.01	75	1	263[3)]	0.3	5.1	0.1
			0.03	150	2	526[3)]	0.6	10.2	0.2
			0.05	300	4	1,052[3)]	1.2	20.4	0.4

Note 1. Estimated value based on literature (Lambert et al., 2007)
Note 2. $AUC_{0\text{-}24\ hr}$
Note 3. Assumption of PK parameters of ISU304 75 IU/kg administered subcutaneously to humans: C_{max} 5.1 IU/dL, T_{max}: 8 h, $t_{1/2}$: 33.8 h, Bioavailability(BA): 31%, The plasma drug concentration decreases exponentially from $C_{max.}$

The initial subcutaneous administration dose of ISU304 has been set as 75 IU/kg (approximately 0.015 mg/kg). This is about 60 times lower than the subcutaneous NOAEL (1 mg/kg/dose, 4,305 IU/kg) and 200 times lower than the intravenous NOAEL (3 mg/kg/dose, 12,915 ~ 14,826 IU/kg) determined in preclinical toxicity studies.
AUC, which indicates systemic exposure and $C_{max,}$ which indicates the toxicity of initial exposure were also reflected in the initial subcutaneous administration dose. The expected AUC value for a subcutaneous 75 IU/kg dose was 263 IU/dL*h (supposing about 30% of AUC compared to intravenous), which was 150 times and 5 times lower than the systemic exposure in rats from

intravenous and subcutaneous administrations respectively (AUC in intravenous administration: 129,581 IU/dL; AUC in subcutaneous administration: 933 IU/dL*h), as determined in a preclinical toxicity study. The expected C_{max} value for a subcutaneous 75 IU/kg dose (5.1 IU/kg: supposed to be 9% of C_{max} value for intravenous administration) was 3,900 times and 10 times lower than C_{max} value for intravenous and subcutaneous NOAEL doses respectively (C_{max} in intravenous dose: 20,061 IU/dL; C_{max} in subcutaneous dose: 49 IU/dL).

In terms of NOAEL, AUC, and C_{max}, the initial subcutaneous administration dose of 75 IU/kg is deemed to be within a safe range. In the actual clinical study, the dose will be doubled, after checking the safety of the earlier dose, to a maximum dose of 150 IU/kg as part of dose escalation study. At the end of the study on each dose, the Data safety Monitoring Committee will evaluate the safety before proceeding to the next planned dose.

Safety in terms of NOAEL

The rationale for the safety of a subcutaneous administration of ISU304 (75 IU/kg) was obtained through a preclinical toxicity study. In terms of dose, 75 IU/kg is approximately 170 ~ 200 times lower than the NOAEL for intravenous administration (on rats and monkeys, 3 mg/kg/dose (12,915 ~ 14,826 IU/kg/dose)) and approximately 60 times lower than the NOAEL for subcutaneous administration (on rats, 1 mg/kg/dose (4,305 IU/kg/dose)) determined in preclinical toxicity studies.
The exposure dose after subcutaneous administration in humans is expected to be 263 IU/dL*h (about 30% of bioavailability), which is about 4,400 times (against monkeys) and 490 times (against rats) lower than the exposure dose confirmed in intravenous administration toxicity studies (1,164,928 IU/dL*hr in monkeys and 129,581 IU/dL*hr in rats). Therefore, it was judged that the safety against the exposure was confirmed in a sufficiently wide range.

Safety in terms of AUC

The bioavailability of subcutaneously administered ISU304 in preclinical studies was 30% or lower (hemophilic dog: 9%, normal mouse: 20%, minipig: 32%) compared to bioavailability in intravenous administration (100%). Furthermore, the bioavailability reported in a PK test for subcutaneous FIX administration on 1 hemophilia patient was also 33% (Lilies et al., 1997). The results of these preclinical and clinical studies were reflected in the initial subcutaneous administration dose.

As the exposure to the drug is lower for subcutaneous administration compared to intravenous, the initial dose of 75 IU/kg, which is used in intravenous administration, is deemed appropriate for the initial subcutaneous dose. When the PK parameters of ISU304 administered subcutaneously were

assumed (C_{max} 5.1 IU/dL, T_{max}: 8 h, $t_{1/2}$: 33.8 h, The plasma drug concentration decreases exponentially from C_{max}.) based on the preclinical and clinical results discussed earlier, the AUC was 263 IU/dL*h, that is 31% bioavailability. This level of exposure is 90 times and 3.5 times lower than the systemic exposure from intravenous and subcutaneous NOAEL doses respectively (AUC in intravenous administration: 129,581 IU/dL*h; AUC in subcutaneous administration: 933 IU/dL*h) in a toxicity study in rats, it was considered that the safety was sufficiently secured.

Safety in terms of C_{max}

The blood concentration of FIX in normal individuals is within the 50 ~ 150 IU/dL range. As a correlation between high FIX concentration and deep vein thrombosis has been reported, this study aims to stay within the normal range for maximum blood concentration (C_{max}) of ISU304 (Lowe et al., 2001). As determined in preclinical animal PK studies, since C_{max}($C_{max}^{SC}/C_{max}^{IV}$ x 100%) in a subcutaneous administration is about 2 ~ 9% lower than C_{max} in an intravenous administration, it is considered safe to administer higher doses compared to the doses in intravenous administration (Table 1.2). The highest C_{max} was observed in minipigs known to be similar to human skin. As this was 9.3% against the C_{max} in intravenous administration, this was used to predict the C_{max} in subcutaneous administration of ISU304. Since the C_{max} in intravenous administration of ISU304 is estimated to be 55 IU/dL, similar to the one of BeneFix, the C_{max} in subcutaneous administration is expected to be 5.1 IU/dL (55 IU/dL x 93%) (Table 1.2). This shows a similar level to the C_{max} value (4.8 IU/kg) in administration of 7.5 IU/kg estimated from the C_{max} value, measured in a hemophilia patient after subcutaneous administration of plasma-derived FXI according to the literature (Lilies et al., 1997). In addition, since it is at least about 10 to 10,000 times lower than the C_{max} values in the NOAEL determined in preclinical toxicity studies (repeated IV dose toxicity study in monkeys: 56,882 IU/dL; repeated IV dose toxicity study in rats: 20,061 IU/dL; repeated SC dose toxicity study in rats: 49 IU/dL), it was deemed that the safety was sufficiently secured in terms of C_{max}.

Determination of blood sample collection period for PK analysis

The determination of the blood sample collection period for PK analysis after subcutaneous administration is not only important for PK analysis but also for subject safety. A longer blood collection period is advantageous for understanding PK, but a lowered blood concentration of FIX (< 1 IU/dL) increases the risk of bleeding. On the other hand, observation over a sufficient period of time after administration is necessary for the PK analysis of this product. Therefore, an appropriate time period which is both sufficient for PK analysis and subject safety has to be determined. For this, we referred to preclinical pharmacokinetic data for ISU304 in subcutaneous administration.

In order to determine the blood collection period after subcutaneous injection, key PK parameters (T_{max}, C_{max}, $t_{1/2}$) were assumed and the blood concentration of the investigational product over time was predicted.

The estimated pharmacokinetic parameters after subcutaneous administration of ISU304 in humans are assumed based on the results of non-clinical pharmacokinetic studies on ISU304 (in a minipig, type B hemophilic dog, hemophilic mouse, and normal mouse), the result of the clinical study on FIX subcutaneously administered to a hemophilia patient (Lilies et al., 1997), the result of clinical study on recombinant FIX administered intravenously (Lambert et al., 2007; Product monograph for BeneFIX, 2012) (Table 1.2). When the same dose (75 IU/kg) of ISU304 and BeneFIX were administered to hemophilia mice, it showed a similar level of C_{max} (ISU304: 22.4 IU/dL; BeneFIX: 22.7 IU/kg) and half-life (ISU304: 12.9 h; BeneFIX: 11.2 h). Based on this preclinical pharmacokinetic study, it was expected that the administration of the similar dose of ISU304 and BeneFIX in hemophilia patients would show similar C_{max} and half-life ($t_{1/2}$). C_{max} and half-life of BeneFIX administered to type B hemophilia patients are known to be 55 IU/dL and 18.8 hours, respectively (Lambert et al., 2007; Monograph for BeneFIX, 2012). Therefore, the C_{max} of ISU304 administered intravenously (75 IU/kg) was set based on the C_{max} (55 IU/dL) of BeneFix administered intravenously.

When a drug is administered subcutaneously, the plasma concentration of the drug is affected by the distribution rate from subcutaneous tissue to blood and the elimination rate from plasma. Due to the differences in structural and physiological characteristics of subcutaneous tissues between humans and animals, there are limitations in predicting pharmacokinetics of subcutaneous administration to humans based on the results from non-clinical studies. Despite the limitations, the subcutaneous tissues of a pig/mini-pig are more similar to the ones in humans than other animals, therefore, it was suggested that they are more suitable for subcutaneous test for the biological products (Rose et al., 1977; Richter et al., 2012; Zheng Y et al., 2012). The drug distribution rate depends on the structure of the subcutaneous tissue, which affects the drug distribution patterns (Richter et al., 2012). Thus, the distribution patterns of biological drug administered subcutaneously in pigs and minipigs will be more similar to the ones in humans, when compared to the ones in rodents. The C_{max} value after subcutaneous administration was assumed in order to model pharmacokinetics of subcutaneous administration and the C_{max} value after subcutaneous administration in a minipig, in which the rate of drug distribution from veins to subcutaneous tissues is considered similar to one in humans due to the structural similarity in subcutaneous tissues, was used as a reference. C_{max} after subcutaneous administration in minipig was 9.3% of C_{max} after intravenous administration. Based on this, the C_{max}

value after subcutaneous administration of ISU304 in a human was estimated to be 5.1 IU/dL (C_{max}^{IV} x 9.3%=55 IU/dL x 9.3%).

Table 1.2 Estimated C_{max}, $t_{1/2}$, T_{max} and AUC after single IV/SC administration of ISU304 75 IU/kg

Dose 75 IU/kg[1)]	C_{max} (IU/dL)			$t_{1/2}$ (h)			T_{max} (h)	AUC (IU/dL*h)		
	C_{max}^{IV}	C_{max}^{SC}	IV vs SC C_{max} (%)	$t_{1/2}^{IV}$	$t_{1/2}^{SC}$	IV vs SC $t_{1/2}$ Ratio (fold)	T_{max}^{SC}	AUC^{IV}	AUC^{SC}	BA (%)
ISU304: Human (estimated)[2)]	**55**	**5.1**	**9.3**	**18.8**	**33.8**	**1.8**	**8**	**851**	**263**	**31**
BeneFIX: Human[3)]	55	ND	ND	18.8	ND	ND	ND	851	ND	ND
pdFIX: Human[4)]	ND	4.3	ND	ND	ND	ND	8	ND	ND	33
ISU304: Minipig[5)]	56	5.2	9.3	11.2	28.1	2.5	6 - 8	555	180	32
ISU304: Type B hemophilic dog[6)]	99	2.8	2.8	51.2	135.4	2.6	48 ~ 72	3,818	360	9
ISU304: Hemophilic mouse [7)]	22	0.9	4.0	12.3	ND	ND	6 - 24	229	ND	ND
ISU304: Normal mouse [8)]	20	1.1	5.6	9.6	17.5	1.8	8	191	38	20

***** Non-clinical results used to predict pharmacokinetic parameters in humans are shown in gray cells.
1) PK parameters were calculated proportionally, assuming that a FIX product was administered at a dose of 75 IU/kg.
2) Estimated PK parameters of ISU304 in human
3) Lambert et al., 2007, Monograph for BeneFIX, 2012;
4) Liles et al., 1997
5-8) Result of non-clinical PK study on ISU304

When a drug is administered subcutaneously, subcutaneous tissues play a role as a drug reservoir that gradually releases the drug to plasma. This results in a different pharmacokinetic profile from intravenous administration and a big difference in a half-life. Since the drug enters blood from subcutaneous tissues and at the same time the drug degradation occurs in blood, it shows the same aspect like the half-life of the drug is increased. In a non-clinical study on subcutaneous injection, the half-life in groups of subcutaneous administration was increased by 1.8 ~ 2.6 times (normal mouse: 1.8 times; mini-pig: 2.5 times; type B hemophilic dog: 2.6 times) depending on the animal species, compared to the groups of intravenous administration. Since the increase in half-life varies by animal species, to apply the value directly to the estimation of increase in half-life in humans. However, considering the safety of clinical study, the half-life ($t_{1/2}$) was calculated with an assumption of the minimum increase in the half-life (1.8 times) ($t_{1/2}^{SC} = t_{1/2}^{IV}$ x 1.8). It was also assumed that the drug administered subcutaneously decreased exponentially from the C_{max}. Bioavailability calculated after subcutaneous administration based on this assumption was 31%, similar to the bioavailability (33%) of pdFIX administered subcutaneously in humans. .

The drug concentration-time graph obtained with these assumptions is presented in Fig 1.2. C_{max} was approximately 5 IU/kg for Cohort 2, which is planned to receive a subcutaneous administration of 75 IU/kg of ISU304, and the plasma activity was predicted to drop to 1 IU/dL after about 3 days (88 hours). Therefore, the blood collection period for Cohort 2 was set as 3 days. Although changes in drug concentration should be tracked for duration about 3 times the half-life (120 hours or 5 days) in PK analysis, the blood collection period was set as 3 days to ensure safety. At initial stage of study protocol development, single administration of ISU304 300 IU/kg was considered for Cohort 4 with the below rationale (After conducting Cohort 1 to Cohort 3, Cohort 4 was deleted based on PK/PD results and dose of Cohort 5 was changed to ISU304 150 IU/kg). C_{max} for Cohort 3 and Cohort 4 which will receive subcutaneous ISU304 doses of 150 IU/kg and 300 IU/kg each, was 10 IU/kg and 20IU/dL respectively. Time taken before plasma activity fell to 1 IU/dL was 124 hours (5 days) and 156 hours (6 days) respectively. The blood collection periods for these two cohorts are identical at 5 days, in order to allow comparison between the groups. As the blood collection period (120 hours) is longer than 3 times the expected half-life (34 hours), this length is deemed to be appropriate for PK analysis as well. Even at the highest dose of 300 IU/kg, C_{max} is below the range of normal individuals, thus it was deemed to be appropriate in terms of safety as well.

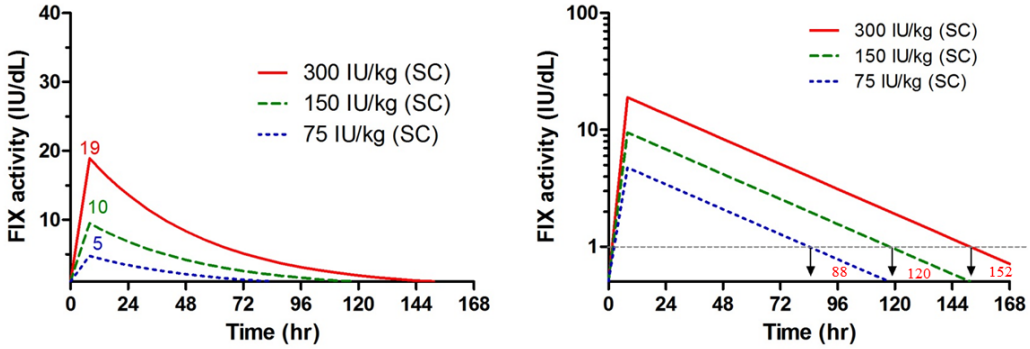


Fig 1.2 Time-FIX concentration graphs by subcutaneous administration dose.
(A) Linear scale, (B) Log scale.
When doses are 75, 150, and 300 IU/kg, time taken for plasma FIX concentration to fall to 1 IU/dL is 88, 124, and 126 hours respectively.

3) Repeated subcutaneous administration

In the preclinical pharmacokinetic study on repeated administration, the drug concentration in plasma was increased due to the drug accumulated in plasma when it was administered 6 times daily. Accumulation ratio calculated using the increase in trough levels (Accumulation ratio = 1st dose

trough level/last dose trough level) was 2.5 times and 13.6 times in minipig and type B hemophilic dog, respectively (Table 1.3). The longer the half-life was, the greater the accumulation ratio was.

Table 1.3 Estimated accumulation ratios after repeated SC administration of ISU304 75 IU/kg in human/animal model

75 IU/kg[1)]	$t_{1/2}^{SC}$	1st dose trough	6th dose trough	Accumulation Ratio (fold)[2)]
ISU304: Human (estimated)[3)]	33.8	3.7	9.0	2.4
ISU304: Minipig [4)]	28.1	3.6	9.2	2.5
ISU304: type B hemophilic dog [5)]	135.4	0.9	11.9	13.6

1) PK parameters were calculated proportionally assuming the FIX administration at a dose of 75 IU/kg.
2) Accumulation ratio = 1st dose trough/last dose trough
3) Estimated value of ISU304 in human
4-5) Values calculated based on the results of non-clinical studies on repeated administration

Using the results from single dose simulations for ISU304, changes in blood concentration of ISU304 from daily subcutaneous administration doses of 75, 150, and 300 IU/kg (single subcutaneous doses administered to Cohorts 2 ~ 4) over 6 days have been simulated (Fig 1.3 and Table 1.4). The accumulation ratio was estimated to be 2.4 times greater when the drug was administered repeatedly 6 times a day.

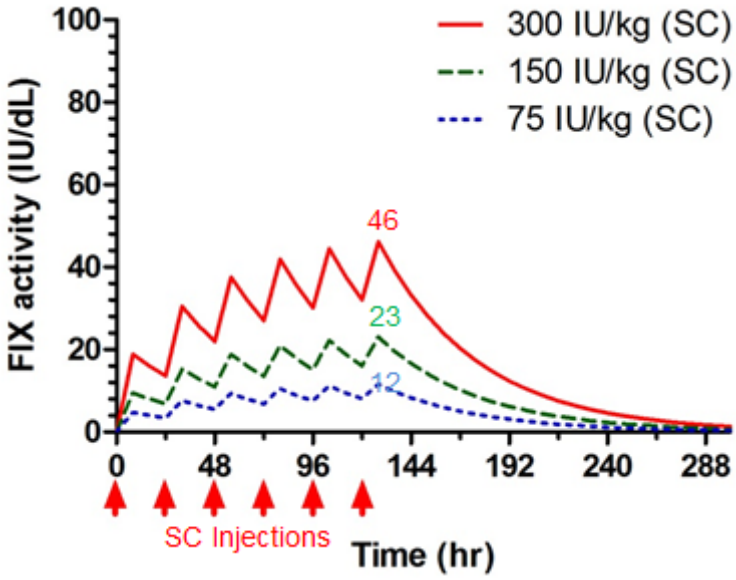


Fig 1.3 Time-FIX concentration graph by dose in repeated subcutaneous administration over 6 days
C_{max} (300 IU/kg: 241 IU/dL; 150 IU/kg: 20 IU/dL; 75 IU/kg: 10 IU/dL) for each dose level presented.

Looking at the results of repeated single subcutaneous doses of 75, 150, and 300 IU/kg over 6 days, the activity concentration in plasma increased daily and C_{max} on day 6 is predicted to be 10, 20, and 41 IU/dL respectively, which falls below the normal range of FIX blood concentration (normal clotting FIX concentration in humans: 50 ~ 150 IU/dL). Based on this, the maximum repeated dose was set as 300 IU/kg. Exposure to the substance over 6 repeated daily subcutaneous administrations in terms of expected AUC and C_{max} is several hundred times (182 ~ 727 times) or several thousand times (3,161 ~ 12,958 times) lower than for a single intravenous infusion to monkeys, thus ensuring a sufficient margin of safety in systemic exposure.

Table 1.4 Safety margin simulation for repeated subcutaneous doses

Simulation	ISU304 (6 repeated daily SC injections)		
	75 IU/kg	**150 IU/kg**	**300 IU/kg**
$AUC_{0\text{-}312h}$ (IU/dL*h)	1,604	3,208	6,416
T_{max}	128	128	128
C_{max}	10	20	41
Margin of safety[1] (Ratio, AUC)	726	363	182
Margin of safety[2] (Ratio, C_{max})	12,958	6,479	3,161
Time for drug concentration to decrease ≤ 1 IU/dL	88 hr (3 day)	124 hr (5 day)	156 hr (6 day)

Note 1. Margin of safety ratio calculations based on value from single IV dose toxicity study on monkeys (1,164,928 IU/dL*hr)
Note 2. Margin of safety ratio calculations based on value from single IV dose toxicity study on monkeys (129,581 IU/dL*hr)

1.2.4 Benefit-Risk Assessment

Based on clinical studies and post-marketing experience of the active control (i.e., BeneFIX), the most serious adverse events are systemic hypersensitivity reactions, including bronchospastic reactions and/or hypotension, anaphylaxis, and the development of high-titer inhibitors necessitating alternative treatments to FIX replacement therapy. Among these, patients with known hypersensitivity, allergy, or anaphylaxis to any FIX product, or patients with inhibitor or family history of inhibitor will be excluded from this clinical study by evaluating exclusion criteria. Additional tests will be conducted in a post-study safety visit 2 weeks (+1 week) after cohort termination to check for inhibitor development in subjects.

Looking at preclinical toxicity animal studies, No AE to the administered product was observed in single intravenous (maximum 10 mg/kg (54,000 IU/kg)), repeated intravenous (maximum 3 mg/kg (12,915 ~ 14,826 IU/kg)), and repeated subcutaneous administrations (maximum 1 mg/kg (4,305 IU/kg)). Therefore, the intravenous injection (75 IU/kg) and subcutaneous injection doses (75, 150, 300 IU/kg) planned in this study is deemed to be relatively safe, considering preclinical data.

Although prophylaxis therapy has been reported to improve patient prognosis in hemophilia B, the ratio of hemophilia B patients who receive continued prophylaxis therapy is not high. As subcutaneous injections offer patients increased convenience and ease in self-administration compared to intravenous, it is expected to allow many patients to use FIX products in continuous prophylaxis therapy. In particular, it is expected to contribute greatly to pediatric treatment.

All things considered, the benefit of treatment in this clinical study is expected to exceed the risk.

1.3 Objective

To investigate the safety, pharmacokinetics and pharmacodynamics of ISU304 (recombinant FIX with increased activity) in previously treated hemophilia B patients.

2 Investigational Products and Other Drugs in the Study

2.1 Investigational Products

2.1.1 Study Drug

- Name: ISU304
- Active ingredient: recombinant factor IX (rFIX) with increased activity
- Volume: 2.0 mg/vial

[redacted]

- Expected indications: Control and prevention of bleeding episodes, and peri-operative management in patients with hemophilia B
- Dosage and administration:

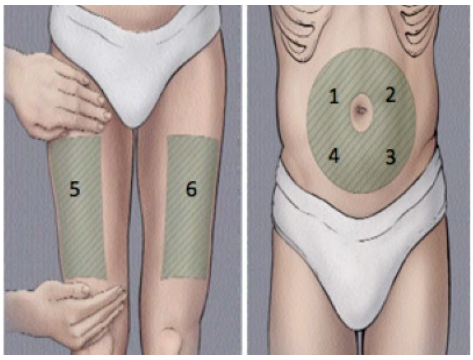
  ISU304 is administered intravenously or subcutaneously by dissolving a lyophilized powder in an injectable formulation. The investigational product dose will be determined according to individual needs, and calculated using the formula below.

  Required FIX quantity (IU) = Weight (kg) × desired FIX increase (IU/kg) in cohort

Intravenous bolus injection

Intravenous administration should be conducted by bolus injection. The investigator should observe to see whether any suspected adverse events (including hypersensitivity) with possible relation to the administration occurs.

Subcutaneous bolus injection



<Administration site>

When administering a 1 ~ 2 mL volume, it is best to use a syringe and needle. The thinner the needle, the better: 28 gauge and 6 mm is recommended. When the volume exceeds 2 mL, a smaller gauge (thicker needle) and slightly longer needle (8 mm) can be used to minimize injection time.

2.1.2 Active Control

- Name: BeneFIX
- Active ingredient: recombinant factor IX (rFIX)
- Volume: 1,000 IU/mL
- Formulation: 0.234% NaCl, 8 mM L-histidine, 0.8% Sucrose, 208 mM Glycine, 0.004% Polysorbate 80, lyophilized injection in a glass vial
- Storage condition/shelf life: below 30℃ (do not freeze)/2 years
- Therapeutic indications: Control and prevention of bleeding episodes, and peri-operative management in patients with hemophilia B
- Dosage and administration: refer to intravenous administration of study drug

2.1.3 Expected Adverse Events Related to the Investigational Product

Based on clinical studies and post-marketing experience of the active control (i.e., BeneFIX), the most serious adverse events are systemic hypersensitivity reactions, including bronchospastic reactions and/or hypotension and anaphylaxis, and the development of high-titer inhibitors necessitating alternative treatments to FIX replacement therapy.

In detail, the most common adverse events observed in clinical studies (> 5%) were headaches, dizziness, nausea, injection site reaction, injection site pain, and skin-related hypersensitivity reactions (e.g., rash, hives). Also, the following post-marketing adverse events have been reported: inadequate FIX recovery, inadequate therapeutic response, inhibitor development, anaphylaxis, angioedema, dyspnea, hypotension, and thrombosis.

Considering the findings from previous studies and the mechanism of study drug, it is expected that the study drug will be associated with similar adverse events to the active control.

2.2 Investigational Product Management

2.2.1 Packaging and Labeling of Investigational Product

Investigational products shall be manufactured in accordance with good manufacturing practice (GMP), and provided to principal investigators and/or sub-investigators who have been delegated study related activities by principal investigators. Each of the following items should be presented on the packaging of investigational products according to Article 69, paragraph 6 of “Regulation on Safety of Medicinal Products, etc. (Ordinance of the Prime Minister No. 1353, 2017.01.04).

1. Mark of “For clinical studies”
2. Code name of product or common name of active ingredient
3. Serial No. and use-by (expiry) date or retest date
4. Method of storage
5. Name and address of a person who receives approval of clinical study protocol
6. Mark of “Not for any purpose other than clinical study”

2.2.2 Handling and Storage of Investigational Products

- Investigational products should be stored at required temperature conditions, and used only on subjects with prescriptions from the principal investigator and/or sub-investigator.
- The sponsor should deliver the investigational products directly to the clinical study pharmacist and get a receipt at the study center after discussion with the principal investigator. The investigational products should be labeled with “For clinical studies”.
- The clinical study pharmacist should ensure that the investigational products are only used in clinical studies.
- During the clinical study, the sponsor should monitor the supply and storage of investigational products, and take appropriate actions when necessary.
- When the clinical study is prematurely terminated or completed, or the investigator has failed to follow the protocol, unused investigational products should be collected and disposed from the study center. The clinical study pharmacist will return the unused investigational products to the sponsor and keep receipt.

2.3 Rescue Drug

The rescue drug used in the study will be the treatment currently being administered to the subject for hemophilia B. The rescue drug must be recorded in the Case Report Form (CRF).

2.4 Concomitant and Prohibited Medications

2.4.1 Concomitant Medications

The following drugs are allowed during the study period.

1) Drugs deemed necessary by the investigator for the management of underlying diseases or adverse events (excluding prohibited medications)

All concomitant medications (including any treatment for the subject's current disease or adverse event) taken during the study will be recorded in the CRF in detail: product name (or active ingredients), purpose of administration, dose, start/stop date, etc.

2.4.2 Prohibited Medications

The following drugs are prohibited during the study period.

1) Anticoagulant or antiplatelet drugs
2) Non-steroidal anti-inflammatory drugs (NSAIDs) that could cause platelet dysfunction (with the exception of Acetaminophen, within domestic legal limits)
3) Immunomodulating agents or immunosuppressants such as α-INF or adrenocortical hormones, and vaccines

If a prohibited medication is required for the treatment of the subject during the study, investigator should immediately terminate the study of the subject.

3 Study Population

3.1 Inclusion Criteria

In order to participate in this study, an individual must meet all of the following criteria.

1) Previously treated male patients with moderate or severe hemophilia B (documented FIX activity ≤ 2% and exposed to any FIX product for ≥ 150 exposure days (estimated) at the time of screening)
2) Patients must be 12 to 65 years old at the time of screening
3) Patients who have discontinued a previously treated FIX product at least 4 days prior to the administration of investigational product
4) HIV negative, or if HIV positive with a CD4 count > 200 μL (documented < 200 particles/μL or ≤ 400,000 copies/mL) at the time of screening
5) Voluntary consent to participate in the study

3.2 Exclusion Criteria

Patients who meet any of the following criteria shall be excluded from the study.

1) Patients with a history or a family history of FIX inhibitors
2) Patients with FIX inhibitors (positive result for BeneFIX or ISU304 from inhibitor tests) at the time of screening
3) Patients who have a history of thromboembolic events (myocardial infarction, cerebrovascular disease, venous thrombosis, etc.)
4) Patients with known hypersensitivity, allergy, or anaphylaxis to any FIX product or hamster protein
5) Patients receiving treatment with a FIX product or a bypass agent within 4 half-lives for the agent used (at least 96 hours) prior to the administration of the investigational product
6) Patients who have been exposed to long-term (exceeding 14 days) administration of immunomodulating agents or immunosuppressants such as α-INF or adrenocortical hormones over the past 3 months or who are currently receiving or planning to receive such treatment during the study period
7) Patients who have been administered vaccines during the period of 6 months prior to the administration of the investigational product or plan to receive vaccines during the study period
8) Patients with any other co-existing bleeding disorder (Von Willebrand disease, etc.)
9) Patients with positive D-dimer results (≥ 0.5 μg/mL) at the time of screening
10) Patients with platelet counts less than 100,000/μL at the time of screening

11) Patients with ALT, AST levels 5 times greater than upper normal limit or total bilirubin, serum creatinine levels 2 times greater than upper normal limit at the time of screening
12) Active hepatitis patients who are HBs Ag positive or anti-HCV Ab positive and require medical treatment at the time of screening
13) Patients scheduled for surgery during the study period
14) Patients participated in another study within 30 days before screening or scheduled to participate in any other study during the study period

3.3 Planned Number of Subjects

At least 12 subjects who meet all of the inclusion criteria and none of the exclusion criteria will be enrolled, including subjects participating the study redundantly.

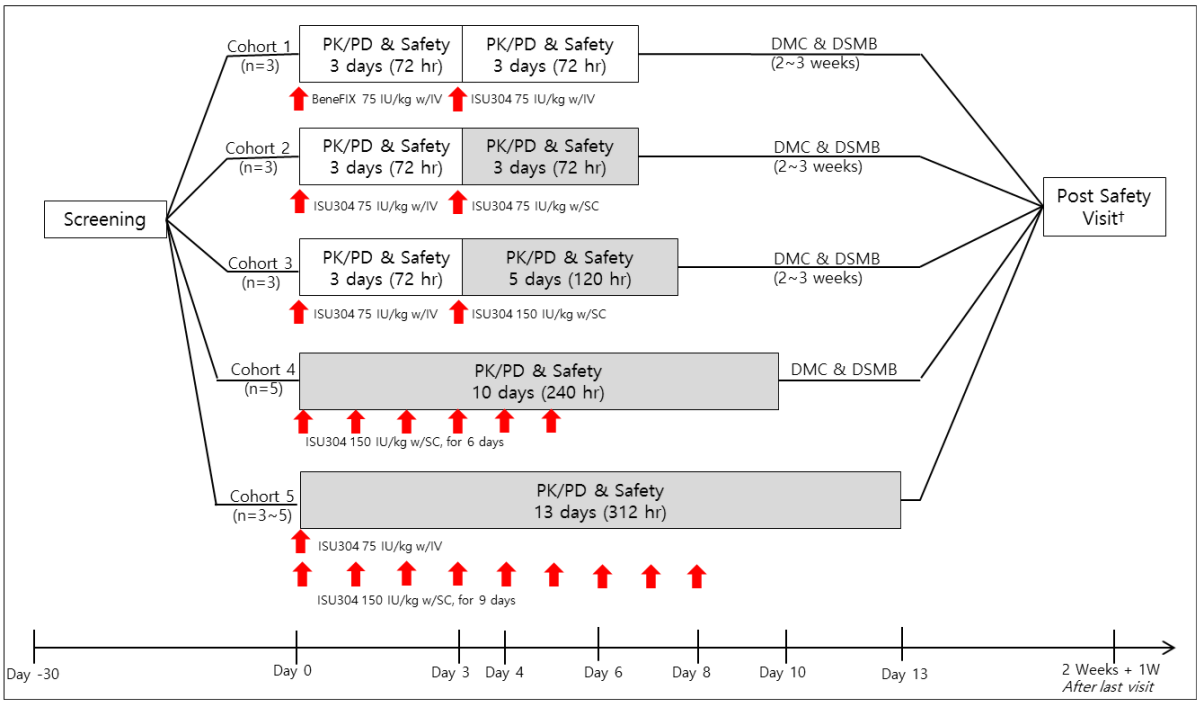
4 Study Method

4.1 Study Design

This study is a phase 1, open-label, multi-center, dose-escalation study to investigate the safety, pharmacokinetics and pharmacodynamics of ISU304 in previously treated hemophilia B patients. All subjects shall give their written consent before participating in this study and only those who meet all of the inclusion criteria and none of the exclusion criteria will be administered the investigational products.

This study is comprised of 5 cohorts. Each cohort will receive an identical intravenous administration of 75 IU/kg, with subcutaneous administrations doubling from 75 IU/kg until 150 IU/kg.

During the study period, a subject may be hospitalized to facilitate the collection of blood samples for pharmacokinetic (PK)/pharmacodynamic (PD) analysis. Hospitalization itself will not be considered as a serious adverse event if no adverse event occurs. PK/PD analysis and safety assessments will be conducted for each cohort. The Data Safety Monitoring Board (DSMB) and Drug Monitoring Committee (DMC) will be operated after the end of Cohorts 1 to 4. These committees will monitor the PK/PD and safety data from each cohort to determine the continuation of next cohort (Cohorts 2 to 5), target dose, and blood sampling period for PK/PD (including timing of collection). It was planned that additional subjects may be enrolled in all cohorts at the early stage of the clinical study, and cohorts may be canceled depending on the results of PK/PD analysis. Since it was confirmed to delete Cohort 4 based on the results of Cohort 3 and to determine 150 IU/kg as the dose of ISU304 in repeated administration. In addition, based on the results of Cohort 4, Cohort 5 was included which administers IV Bolus of ISU304 75 IU/kg followed by daily subcutaneous administration of ISU304 150 IU/kg for 9 days. The clinical study design is as follows.



Administration Point
* PK: Pharmacokinetics; PD: Pharmakodynamics; IV: Intravenous; SC: Subcutaneous
DMC: Drug Monitoring Committee; DSMB: Data Safety Monitoring Board
† Performing the inhibitor test in 2 weeks from the last visit at each cohort (visit window: +1 week)

4.2 Study Duration

Approximately 24 months after the protocol approval from the institutional review boards (IRBs)

4.3 Indication

Previously treated hemophilia B patients

4.4 Administration of Investigational Products and Follow Up Periods

- Cohort 1 (n=3) : 7 days
  Single intravenous administration of BeneFIX (75 IU/kg) with 72 hours of observation, followed by single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation
- Cohort 2 (n=3) : 7 days
  Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (75 IU/kg) with 72 hours of observation
- Cohort 3 (n=3) : 9 days
  Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (150 IU/kg) with 120 hours of observation
-
- Cohort 4 (n=5) : 6 days
  Single subcutaneous administration of ISU304 (150 IU/kg) per day for 6 days with 144 hours of observation
  * At least 5 Subjects including the subjects from Cohorts 1 ~ 3 are expected to participate in Cohort 4.
- Cohort 5 (n=3~5) : 15 days
  Single intravenous administration of ISU304 (75 IU/kg) is followed by subcutaneous ISU304 (150 IU/kg) administration for 9 days with 312 hours of observation

The baseline for all cohorts will be at the time before the intravenous or subcutaneous administration of the investigational products. Additional subjects may be enrolled in all cohorts or cohorts may be canceled depending on the results of PK/PD analysis. 14 days are allowed from the first

administration of the study drug to the second administration in cohorts 1 to 4 depending on the condition of subjects. If bleeding occurs 72 hours after first administration in cohorts 1 to 4 and FIX product is administered as a rescue medication, there must be at least of 4 days of wash-out period from the last administration of FIX.

4.5 Measures to Minimize Bias

4.5.1 Subject Identification Codes

A unique identification code will be assigned to each subject according to the time of signature and used in lieu of the subject's name to protect the subject's identity. The subject identification code will be assigned as follows.

Screening No.: CX-YY-SZZ (X: Cohort No. (1, 2~), YY:Center No. (01, 02 ~), S: first letter of 'Screening', ZZ: Subject serial No. (01, 02 ~))
At the time of screening, a screening No. will be assigned to a subject according to the order of signature.

Registration No.: CX-YY-RZZ (X: Cohort No. (1, 2 ~), Center No. (01, 02 ~), R: first letter of 'Registration', ZZ: Subject serial No. (01, 02 ~))
After the final eligibility decision is made based on the inclusion/exclusion criteria, a registration No. is assigned to a subject prior to the 1st administration of the investigational product.

4.5.2 Blinding

Not applicable

4.5.3 Randomization

Not applicable

5 Study Procedure

5.1 Measurements

5.1.1 Informed Consent

Before participation in this study, a subject will provide written informed consent after receiving a full explanation of the study objective and procedure by the investigator. The investigator will then assign a unique screening number to the subject. The date of the signing of the informed consent form must be recorded in the CRF.

5.1.2 Inclusion/Exclusion Criteria

After written informed consent is obtained, the subject will undergo the screening test. For laboratory test conducted for confirmation of inclusion/exclusion criteria, retest is allowed during the screening period as judged by the Investigator. If not eligible even after the retest, the subject is not allowed to enroll this study. Only subjects who are eligible according to inclusion/exclusion criteria will be treated with the investigational products.

5.1.3 Demographics

A subject's demographic data (i.e., gender, date of birth, age, weight (kg), and height (cm)) will be recorded at the time of screening.

5.1.4 Hemophilia B Status

The subject's detailed information about documentation of the lowest FIX activity, year of diagnosis, family history of hemophilia B, history and family history of FIX inhibitors, and previous FIX treatment history (drugs administered and administration period) at the time of screening are recorded.

5.1.5 Medical and Medication History

Subject's past (within 3 months prior to screening) or current medical history and medication history (within 3 months prior to screening, within 6 months for vaccines) will be obtained by interview and/or medical records at the time of screening.

5.1.6 Physical Examination

The following system organs are assessed at the time of screening, right before investigational product administration, 6 hours after investigational product administration, all planned visits after administration, interim visits, and end of a cohort (as there is no interim visit for Cohort 4 and Cohort 5, this is replaced by the date of the 1st investigational product administration, and all planned visits after administration is set to 24 hours after each investigational product administration): Skin/extremities, HEENT (head, eye, ear, nose, and throat), chest & lung, abdomen, musculoskeletal system, nervous system, and genitourinary system. Also, tolerability of subcutaneous injection (excluding Cohort 1), local reactions in administration site, and systemic reactions are assessed after administration of investigational products.

If a clinically significant abnormal finding is observed in the physical examination, it should be recorded as current medical history (at the time of screening) or an adverse event (after the administration of investigational products) on the CRF.

5.1.7 Vital Signs

The subject's vital signs (systolic and diastolic blood pressure (mmHg), pulse rate (bpm), respiration rate (bpm), and body temperature (℃)) should be measured and recorded at the time of screening, right before investigational product administration, 6 hours after investigational product administration, all planned visits after administration, interim visits, and end of a cohort (as there is no interim visit for Cohort 4 and Cohort 5, this is replaced by the date of the 1st investigational product administration, and all planned visits after administration is set to 24 hours after each investigational product administration). Blood pressure is measured in a sitting position after resting sufficiently for at least 5 minutes.

If a clinically significant abnormal finding is observed during the examination of vital signs, it should be recorded as current medical history (at the time of screening) or an adverse event (after the administration of investigational products) on the CRF.

5.1.8 Electrocardiogram

Electrocardiogram is obtained at the time of screening, 1st day of hospitalization, interim visits, 2nd hospitalization, and end of a cohort (as there is no interim visit for Cohort 4 and Cohort 5, this is replaced by the date of the 1st investigational product administration). The actual recording date/time, PR, QRS, QT, corrected QT intervals, and investigator's assessment will be recorded in the CRF. Results are classified into followings: 1) Normal, 2) Abnormal, not clinically significant, or 3) Abnormal, clinically significant.

If a clinically significant abnormal finding in electrocardiogram (e.g., abnormal change in QT interval) is observed, it should be recorded as current medical history (at the time of screening) or an adverse event (after the administration of investigational products) on the CRF.

5.1.9 Laboratory Tests

Laboratory tests are carried out at the central laboratory (SCL; Seoul Clinical Laboratories, KR). However, the tests requiring analysis within 24 hours (Hematology, Urine tests) can be conducted at each study site. In special circumstances (blood sampling on Saturday and on the day before holidays, for the purpose of checking adverse events), the laboratory tests may be carried out separately at each study sites, and the test results shall be recorded in the CRF.

For laboratory test conducted for confirmation of inclusion/exclusion criteria, retest is allowed during the screening period as judged by the Investigator. If not eligible even after the retest, the subject is not allowed to enroll this study.

Hematology, blood chemistry, serology, and urinalysis are conducted at the time of screening, 1st day of hospitalization, interim visits, 2nd hospitalization, and end of a cohort (as there are no interim visits or hospitalizations for Cohort 4 and Cohort 5, they are replaced by the date of the 1st investigational product administration).

- Hematology : RBC, WBC with differential count (Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil), Hemoglobin, Hematocrit, Platelet count, CD4 count (Screening only)
- Blood chemistry : Total protein, Albumin, Creatinine, BUN, Uric acid, Total bilirubin, AST (SGOT), ALT (SGPT), γ-GT
- Serology (Screening only) : anti-HBV (HBs Ag), anti-HCV (Anti-HCV Ab), anti-HIV (Anti-HIV Ab)

- Urinalysis : pH, Protein, Glucose, Urobilinogen, Microscopic exam (if abnormal dipstick test): RBC, WBC

Blood coagulation test is only conducted at the time of screening (only D-dimer test is conducted), 1st and 2nd hospitalization days, 6 hours after investigational product administration, all planned visits after administration, interim visits, and end of a cohort (as there are no interim visits or hospitalizations for Cohort 4 and Cohort 5, they are replaced by the date of the 1st investigational product administration, and all planned visits after administration is set to 24 hours after each investigational product administration). Blood coagulation test will include below.

- Blood coagulation : aPTT*, PT international normalized ratio (INR), PT sec, Thrombin-antithrombin complex (TAT), D-dimer, Prothrombin fragment 1+2 (F1+2), Fibrinogen

  *aPTT is a pharmacodynamic endpoint which shall be analyzed additionally in a different central laboratory (HTI; Haematologic Technologies Inc., USA), and the timing of blood sample collection is in 5.1.12.

A clinically significant abnormal laboratory test result after investigational product administration shall be considered an adverse event.

Sample storage and disposal will be carried out according to central laboratory's recommended procedure.

Refer to 5.1.12 for the timing of blood collection and the amount of blood required per visit.

5.1.10 FIX Inhibitor and Anti-drug Antibody Test

FIX inhibitor (or neutralizing antibody (NAb)) and anti-drug antibody test as well as a separate Bethesda assay shall be conducted in the central laboratories (inhibitor test, anti-drug antibody test: HTI, Bethesda assay: SCL) at the time of screening, 1st hospitalization, interim visit, 2nd hospitalization, and end of a cohort (with the exception of Bethesda assay at the time of screening). Tests for inhibitors and anti-drug antibodies to ISU304 and BeneFIX shall be conducted using a method developed by the sponsor.

Inhibitors to ISU304 and BeneFIX are detected through the Nijmegen method. When there is a positive result(>0.6 $BU)_{(14)}$, a confirmatory test will be carried out. The inhibitor titer shall be recorded in Bethesda units (BUs). Anti-drug antibodies to ISU304 and BeneFIX are detected through a direct binding enzyme-linked immunosorbent assay (ELISA).

5.1.11 Administration of Investigational Product

Subjects will be treated with the investigational products according to the planned dosing schedule for each cohort. The date/time, dose, and route of administration are to be recorded in the CRF.

5.1.12 Blood Collection

For PK/PD analysis, the subject's blood samples will be collected according to defined time points below: The blood collection volumes for each visit by cohort are listed below, and the actual time of blood collection shall be recorded and used in the analysis. However, for the subject cannot visit, the Investigator can visit to collect the sample (select one among blood or saliva, oral epithelial cells) for Factor 9 gene mutation test.

Blood collection volume for laboratory tests and pharmacokinetic/pharmacodynamic analysis (estimated)

		Screening	1st/2nd Hospitalization	Administration of investigational product and timing of blood collection for PK/PD analysis										Interim visit/ End of a cohort	Post-study safety visit	Blood collection volume per cohort (mL)
Hours after administration	Intravenous administration	N/A	N/A	Before administration	0, 0.25, 0.5, 1, 3	6	9	24	48	72	96	120	144	NA	NA	
	Subcutaneous administration				1, 2, 4	6	8, 10, 12									
Blood collection volume per visit (mL)†		**20.7**	**37.9**	**32.4**				**11.4**	**11.4**	**11.4**	**8.4**	**11.4**	**11.4**	**37.9**	**21.5**	-
Laboratory tests (mL)																
Complete blood count		8	8	-	-		-	-	-	-	-	-	-	8	-	-
Blood coagulation		2.7[a]	8.4	-	-	8.4	-	8.4	8.4	8.4	8.4[b]	8.4	8.4[b]	8.4	-	
Bethesda assay			1.5	-	-		-							1.5	1.5	
Anti-drug antibodies/neutralizing antibodies to investigational product		10	20	-	-		-							20	20	
IgG, IgE, Factor 9 gene mutation test[d], HLA typing and other IR (immune response) genes (If required)		-	-	-	-	-	-	-	-	-	-	-	-	-	3	
PK/PD analysis (mL)																
Cohort 1	BeneFIX 75 IU/kg w/IV	3	-	3	3 (per time)	3	3 (per time)	3	3	3	-	-	-	-	-	310.2
	ISU304 75 IU/kg w/IV	-	-	3	3 (per time)	3	3 (per time)	3	3	3	-	-	-	-	-	
Cohort 2	ISU304 75 IU/kg w/IV	3	-	3	3 (per time)	3	3 (per time)	3	3	3	-	-	-	-	-	310.2
	ISU304 75 IU/kg w/SC	-	-	3	3 (per time)	3	3 (per time)	3	3	3	-	-	-	-	-	
Cohort 3	ISU304 75 IU/kg w/IV	3	-	3	3 (per time)	3	3 (per time)	3	3	3	-	-	-	-	-	321.6
	ISU304 150 IU/kg w/SC	-	-	3	3 (per time)	3	3 (per time)	3	3	3	-	3	-	-	-	
Cohort 4[e]	ISU304 150 IU/kg w/SC	-	-	3 (per time)	-		-	3		3		3		-	-	145.0
Cohort 5[c]	ISU304 75 IU/kg w/IV	-	-	3	3(per time)‡											166.0
	ISU304 150 IU/kg w/SC	-	-	3(per time)♀	-	-	-	3	-	3		3				

† – Volume of collected blood from a Cohort 5 subject is 11.4 mL at 96 hours after 1st administration (visit 5) and 14.4 mL at 120 hours after 1st administration (visit 6).

‡ – At the first administration day of Cohort 5, total 3 PK/PD sample collections, right before IV administration, 0 min. and 0.5 min. will be done.

♀ – The first subcutaneous administration is performed within 30 min. of IV administration and PK/PD sampling is done right before subcutaneous administration for 9 days.

a – Only D-dimer is assessed.

b – Only for Cohort 4 and Cohort 5

c- Only perform the neutralizing antibody test

d- Sample collection can be performed through oral epithelial cells, saliva or blood.

e – Blood collection for PK/PD analysis in Cohort 4 take place right before each administration, 6 hours after 1st administration, 6 hours after 6th administration, and 24 hours after 6th administration.

5.1.13 Pharmacokinetic/ Pharmacodynamic Endpoints

The following PK endpoints will be estimated and analyzed at the central laboratory (HTI) for each subject: observed maximum plasma concentration (C_{max}), terminal phase elimination half-life ($t_{1/2}$), total plasma clearance (CL), volume of distribution at steady state (V_{ss}), area under curve (AUC; $AUC_{(0-t)}$ and $AUC_{(0-inf)}$), mean residence time (MRT), incremental recovery (K), time to maximum plasma concentration (T_{max}) for FIX activity and FIX antigen.

For the PD endpoint, activated partial thromboplastin time (aPTT) will be estimated and analyzed at the central laboratory (HTI, USA).

The subject's blood samples will be collected according to the defined time points below for PK/PD analysis.

- Cohort 1 Right before intravenous administration of BeneFIX , 0(immediately after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min, 24hr±15min, 48hr±120min, 72hr±120min after administration;

  Right before intravenous administration of ISU304, 0(immediately after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min, 24hr±15min, 48hr±120min, 72hr±120min after administration
- Cohort 2 Right before intravenous administration of ISU304, 0(immediately after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min, 24hr±15min, 48hr±120min, 72hr±120min after administration;

  Right before subcutaneous administration of ISU304, 1hr±3min, 2hr±3min, 4hr±5min, 6hr±5min, 8hr±5min, 10hr±5min, 12hr±5min, 24hr±15min, 48hr±120min, 72hr±120min after administration
- Cohort 3 Right before intravenous administration of ISU304, 0(immediately after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min, 24hr±15min, 48hr±120min, 72hr±120min after administration;

  Right before subcutaneous administration of ISU304, 1hr±3min, 2hr±3min, 4hr±5min, 6hr±5min, 8hr±5min, 10hr±5min, 12hr±5min, 24hr±15min, 48hr±120min, 72hr±120min, 120hr±180min after administration

- Cohort 4 Right before 1st subcutaneous administration of ISU304, 1st administration+6hr, right before 2nd subcutaneous administration, right before 3rd subcutaneous administration, right before 4th subcutaneous administration, right before 5th subcutaneous administration, right before 6th subcutaneous administration, 6th administration+6hr, 6th administration+24hr
- Cohort 5 Right before and after intravenous administration of ISU304 75 IU/kg, 30 min. after administration (after 1st subcutaneous administration of ISU304 150 IU/kg), and right before 2nd subcutaneous administration of ISU304 150 IU/kg, right before 3rd subcutaneous administration, right before 4th subcutaneous administration, right before 5th subcutaneous administration, right before 6th subcutaneous administration, right before 7th subcutaneous administration, right before 8th subcutaneous administration, right before 9th subcutaneous administration, 9th subcutaneous administration+24 hr, 9th subcutaneous administration+72 hr, 9th subcutaneous administration+120 hr

5.1.14 Concomitant Medications

A subject's concomitant medications at the time of screening and any changes in medications compared to screening should be recorded in the CRF. Rescue drugs should be recorded separately.

5.1.15 Adverse Events (AEs)

The investigator should collect and review AEs of a subject after administration of the investigational products through interview and/or tests. AEs occurring after the administration of investigational products shall be classified into local, systemic, and other AEs[10, 13]. The adverse events are evaluated using the ruler provided and thermometers at institution for accurate assessment of adverse events.

1) Local AE

All local AEs occurring within 1 hour after the administration of investigational products including pain, tenderness, erythema/redness, urticaria/pruritus, and induration/swelling should be recorded. The severity of the symptoms should also be recorded according to separate AE assessment criteria and the causal relationship with the investigational products should also be evaluated. (Appendix 4. Table – Assessment of Local Adverse Events)

2) Systemic AE

All systemic AEs occurring within 1 hour after the administration of investigational products including fever, fatigue/malaise, chill/pyrexia, , headache, myalgia, arthritis(arthralgia), decreased appetite/loss of appetite, diarrhea, vomiting, constipation, abdominal pain, mucosal skin reaction/rash, vasovagal syncope, dizziness, cough, acute bronchospasm, dyspnea, and hypersensitivity should be recorded according to the AE assessment criteria and the causal relationship with the investigational products should also be evaluated. The severity of the symptoms should also be recorded according to separate AE assessment criteria and the causal relationship with the investigational products should also be evaluated. (Appendix 5. Table – Assessment of Systemic Adverse Events)

3) Other AE

All AEs other than local or systemic AEs occurring after the administration of investigational products should be recorded. The severity of the symptoms should also be recorded according to AE assessment criteria and the causal relationship with the investigational products should also be evaluated.

Adverse events of special interest (AESIs) should be collected and analyzed separately. Following AESIs will be monitored.

- Thrombosis/embolism
- Spontaneous bleeding
- Inhibitor generation/seroconversion
- Anaphylaxis

The source document on an AE should include the following: onset/end date, time (only for AESI), severity, outcome, action taken to investigational products, causal relationship with investigational products, causal relationship with other drugs, treatment given for AE, and seriousness.

In the occurrence of AEs related to blood coagulation, a blood coagulation test shall be carried out and the results will be recorded in the CRF.

5.2 Detailed Procedure for Each Visit

Subjects of Cohorts 1 to 3 will be hospitalized on the day of investigational product administration to facilitate collection of blood samples for pharmacokinetic/pharmacodynamic (PK/PD) analysis. Cohort 4 and Cohort 5 subjects will be administered the investigational product on outpatient visits to the hospital, without the need for hospitalization. However, hospitalization or the prolongation of hospitalization can be conducted to facilitate the collection of samples for PK/PD analysis. Hospitalization itself shall not be considered as an SAE if no AE occurs during the hospitalization.

5.2.1 Cohort 1 and Cohort 2

Cohort 1 will be intravenously administered 75 IU/kg of BeneFIX and blood samples will be collected over 3 days (72 hours) thereafter for PK/PD analysis in the 1st administration period. Then, 75 IU/kg of ISU304 will be intravenously administered for the 2nd administration period and blood samples will be collected over 3 days (72 hours) thereafter for PK/PD analysis as in the 1st administration period. Cohort 2 shall follow the identical procedures as Cohort 1 but will be administered different investigational products. Then, 75 IU/kg of ISU304 will be intravenously administered in the 1st administration period, followed by a subcutaneous administration of 75 IU/kg of ISU304 in the 2nd administration period. The detailed procedures for the respective visits are as follows.

5.2.1.1 Screening Visit (Day -30 to 0)

The screening visit should be conducted within -30 days to the administration date. It should be scheduled in consideration of the fact that some tests for the inclusion/exclusion criteria will be conducted in an overseas central laboratory. The following is the procedure for the Screening Visit.

① Obtaining written informed consent from the subject
② Assigning screening no.
③ Collecting demographic data, hemophilia B status, medical and medication history (determining eligibility based on inclusion/exclusion criteria)
④ Physical examination, assessment of vital signs and electrocardiogram
⑤ Blood/urine sample collection for laboratory tests (only D-dimer test is conducted for blood coagulation, retest is allowed during screening period as judged by the Investigator) and FIX inhibitor test

5.2.1.2 Visit 1 (Day -1 to 1) - 1st Hospitalization and Registration

Visit 1 is for the 1st administration of the investigational product, and the subject is hospitalized for a day to conduct this visit. The subject is recommended to visit the hospital to be hospitalized on the day before the administration. As the timing of the 1st administration is identical to the time that the subject will have to visit the hospital on subsequent visits, it is important to set the time of the 1st administration with consideration for the subject's schedule for the next 4 days. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 1.

1st hospitalization (Day -1 to 0)

① Assigning registration no. after final eligibility assessment
② Hospitalization procedure for subject
③ Checking for additional medical and medication history
④ Electrocardiogram
⑤ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay

1st administration and blood collection for PK/PD analysis

① Checking for additional medical history and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product
④ Administration of investigational product
For Cohort 1, BeneFIX 75 IU/kg, intravenous administration
For Cohort 2, ISU304 75 IU/kg, intravenous administration
⑤ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑥ Blood sample collection for PK/PD analysis
0(right after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min
⑦ Physical examination, assessment of vital signs, sample collection for blood coagulation test 6 hours after administration

1st discharge (Day 1)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
24hr±15min

④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit
⑥ Discharge procedure for subject

5.2.1.3 Visit 2 (Day 2) - Outpatient Visit

The subject shall be instructed and reminded to visit the hospital within 48hr±120min from the time of the 1st investigational product administration for Visit 2. The following is the procedure for Visit 2.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
48hr±120min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.1.4 Visit 3 (Day 3) - Interim Visit (termination of 1st administration)

The subject shall be instructed and reminded to visit the hospital within 72hr±120min from the time of the 1st investigational product administration for Visit 3. Visit 3 is an interim visit marking the termination of the 1st administration period. The following is the procedure for Visit 3.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
72hr±120min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay
⑤ Notification of visit schedule and scheduling of next visit

5.2.1.5 Visit 4 (Day 3 to 5) - 2nd Hospitalization

Visit 4 is for the 2nd administration of the study drug, and the subject is hospitalized for a day as in Visit 1. The subject is recommended to visit the hospital to be hospitalized on the day before the administration. The timing of the 2nd administration is also identical to the time that the subject will have to visit the hospital on subsequent visits. Thus the time of the 2nd administration should be determined with consideration for the subject's schedule.

An interval of at least 4 days is required between the administration of the 1st and 2nd doses. The investigator shall schedule the visit in consideration of this interval. Depending on the schedule, the procedure for Visits 3 and 4 could be conducted on the same day. Should this be the case, all procedures for the respective visits must be carried out without fail. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 4.

2nd hospitalization (Day 3 to 4)

① Hospitalization procedure for subject
② Checking for AEs and concomitant medications
③ Electrocardiogram
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay

2nd administration and blood collection for PK/PD analysis (Day 4)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product
④ Administration of investigational product
For Cohort 1, ISU304 75 IU/kg, intravenous administration
For Cohort 2, ISU304 75 IU/kg, subcutaneous administration
⑤ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑥ Blood sample collection for PK/PD analysis
For Cohort 1,
: 0(right after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min
For Cohort 2,
: 1hr±3min, 2hr±3min, 4hr±5min, 6hr±5min, 8hr±5min, 10hr±5min, 12hr±5min
⑦ Physical examination, assessment of vital signs, sample collection for blood coagulation test 6 hours after administration

2nd discharge (Day 5)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
24hr±15min

④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit
⑥ Discharge procedure for subject

5.2.1.6 Visit 5 (Day 2) - Outpatient Visit

The subject shall be instructed and reminded to visit the hospital within 48hr±120min from the time of the 2nd investigational product administration for Visit 5. The following is the procedure for Visit 5.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
48hr±120min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.1.7 Visit 6 (Day 7) - End of a Cohort

The subject shall be instructed and reminded to visit the hospital within 72hr±120min from the time of the 2nd investigational product administration for Visit 6. The following is the procedure for Visit 6.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
72hr±120min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay
⑤ Termination of cohort and notification of post-study safety visit

5.2.2 Cohort 3

Cohort 3 will be intravenously administered 75 IU/kg of ISU304 and blood samples will be collected over 3 days (72 hours) thereafter for PK/PD analysis in the 1st administration period. Then, 150 IU/kg of ISU304 will be subcutaneously administered for the 2nd administration period and blood samples will be collected over 5 days (120 hours) thereafter for PK/PD analysis. The detailed procedures for the respective visits are as follows.

5.2.2.1 Screening Visit (Day -30 to 0)

The screening visit should be conducted within -30 days to the administration date. It should be scheduled in consideration of the fact that some tests for the inclusion/exclusion criteria will be conducted in an overseas central laboratory. The following is the procedure for the Screening Visit.

① Obtaining written informed consent from the subject
② Assigning screening no.
③ Collecting demographic data, hemophilia B status, medical and medication history (determining eligibility based on inclusion/exclusion criteria)
④ Physical examination, assessment of vital signs and electrocardiogram
⑤ Blood/urine sample collection for laboratory tests (only D-dimer test is conducted for blood coagulation, retest is allowed during screening period as judged by the Investigator), FIX inhibitor test and anti-drug antibody test

5.2.2.2 Visit 1 (Day -1 to 1) - 1st Hospitalization and Registration

Visit 1 is for the 1st administration of the investigational product, and the subject is hospitalized for a day to conduct this visit. The subject is recommended to visit the hospital to be hospitalized on the day before the administration. As the timing of the 1st administration is identical to the time that the subject will have to visit the hospital on subsequent visits, it is important to set the time of the 1st administration with consideration for the subject's schedule for the next 4 days. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 1.

1st hospitalization (Day -1 to 0)

① Assigning registration no. after final eligibility assessment
② Hospitalization procedure for subject
③ Checking for additional medical and medication history
④ Electrocardiogram
⑤ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay

1st administration and blood collection for PK/PD analysis

① Checking for additional medical history and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product

④ Intravenous administration of 75 IU/kg of ISU304
⑤ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑥ Blood sample collection for PK/PD analysis
0(right after administration)hr±1min, 0.25hr±3min, 0.5hr±3min, 1hr±3min, 3hr±5min, 6hr±5min, 9hr±5min
⑦ Physical examination, assessment of vital signs, sample collection for blood coagulation test 6 hours after administration

1st discharge (Day 1)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
24hr±15min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit
⑥ Discharge procedure for subject

5.2.2.3 Visit 2 (Day 2) - Outpatient Visit

The subject shall be instructed and reminded to visit the hospital within 48hr±120min from the time of the 1st investigational product administration for Visit 2. The following is the procedure for Visit 2.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
48hr±120min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.2.4 Visit 3 (Day 3) - Interim Visit (termination of 1st administration)

The subject shall be instructed and reminded to visit the hospital within 72hr±120min from the time of the 1st investigational product administration for Visit 3. Visit 3 is an interim visit marking the termination of the 1st administration period. The following is the procedure for Visit 3.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram

③ Blood sample collection for PK/PD analysis
72hr±120min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay
⑤ Notification of visit schedule and scheduling of next visit

5.2.2.5 Visit 4 (Day 3 to 5) - 2nd Hospitalization

Visit 4 is for the 2nd administration of the study drug, and the subject is hospitalized for a day as in Visit 1. The subject is recommended to visit the hospital to be hospitalized on the day before the administration. The timing of the 2nd administration is also identical to the time that the subject will have to visit the hospital on subsequent visits. Thus the time of the 2nd administration should be determined with consideration for the subject's schedule.

An interval of at least 4 days is required between the administration of the 1st and 2nd doses. The investigator shall schedule the visit in consideration of this interval. Depending on the schedule, the procedure for Visits 3 and 4 could be conducted on the same day. Should this be the case, all procedures for the respective visits must be carried out without fail. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 4.

2nd hospitalization (Day 3 to 4)

① Hospitalization procedure for subject
② Checking for AEs and concomitant medications
③ Electrocardiogram
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay

2nd administration and blood collection for PK/PD analysis (Day 4)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product
④ Administration of investigational product
For Cohort 3, ISU304 150 IU/kg, subcutaneous administration

⑤ Checking for AEs at least 30 min to 1 hr after investigational product administration

⑥ Blood sample collection for PK/PD analysis
1hr±3min, 2hr±3min, 4hr±5min, 6hr±5min, 8hr±5min, 10hr±5min, 12hr±5min

⑦ Physical examination, assessment of vital signs, sample collection for blood coagulation test 6 hours after administration

2nd discharge (Day 5)

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
24hr±15min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit
⑥ Discharge procedure for subject

5.2.2.6 Visit 5 (Day 6) and Visit 6 (Day 7) - Outpatient Visit

The subject shall be instructed and reminded to visit the hospital within 48hr±120min (for Visit 5), and within 72hr±120min from the time of the 2nd investigational product administration. The following is the procedure for Visits 5 and 6.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis
48hr±120min, 72hr±120min
④ Sample collection for blood coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.2.7 Visit 7 (Day 9) - End of a Cohort

The subject shall be instructed and reminded to visit the hospital within 120hr±180min from the time of the 2nd investigational product administration for Visit 7. The following is the procedure for Visit 7.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
120hr±180min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test,

and Bethesda assay

⑤ Termination of cohort and notification of post-study safety visit

5.2.3 Cohort 4

Cohort 4 will be subcutaneously administered 150 IU/kg of ISU304 once a day for 6 days, and blood samples will be collected over 240 hours thereafter for PK/PD analysis. The dose for Cohort 4 may be modified according to PK/PD and safety data from Cohorts 1 to 3. The detailed procedures for the respective visits are as follows.

5.2.3.1 Screening Visit (Day -30 to 0)

The screening visit should be conducted within -30 days to the administration date. It should be scheduled in consideration of the fact that some tests for the inclusion/exclusion criteria will be conducted in an overseas central laboratory. The following is the procedure for the Screening Visit.

① Obtaining written informed consent from the subject
② Assigning screening no.
③ Collecting demographic data, hemophilia B status, medical and medication history (determining eligibility based on inclusion/exclusion criteria)
④ Physical examination, assessment of vital signs and electrocardiogram
⑤ Blood/urine sample collection for laboratory tests (only D-dimer test is conducted for blood coagulation, retest is allowed during screening period as judged by the Investigator), FIX inhibitor test and anti-drug antibody test

5.2.3.2 Visit 1 (Day 0) - Registration and Administration of Investigational Product

Visit 1 is for the 1st administration of investigational product and the timing of the 1st administration is identical to the time that the subject will have to visit the hospital on subsequent visits. So, it is important to set the time of the 1st administration with consideration for the subject's schedule in the next 6 days. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 1.

① Assigning registration no. after final eligibility assessment
② Checking for additional medical history and concomitant medications
③ Physical examination, assessment of vital signs and electrocardiogram
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test and Bethesda assay before administration of investigational product

⑤ Subcutaneous administration of 150 IU/kg of ISU304
⑥ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑦ Notification of visit schedule and scheduling of next visit

5.2.3.3 Visit 2 (Day 1) - Visit 6 (Day 5) - Repeated Administration of Investigational Product

The subject shall be instructed and reminded to visit the hospital in 24hr±15min intervals starting from the time of the 1st investigational product administration for Visits 2 to 6. The following is the procedure for Visits 2 to 6.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product
④ Sample collection for coagulation test
⑤ Subcutaneous administration of 150 IU/kg of ISU304
⑥ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑦ Blood sample collection for PK/PD analysis (only on Visit 6)
6hr±5min
⑧ Physical examination, assessment of vital signs, sample collection for blood coagulation test 6 hours after administration
⑨ Notification of visit schedule and scheduling of next visit

5.2.3.4 Visit 7 (Day 6)

The subject shall be instructed and reminded to visit the hospital within 24hr±15min from the time of Visit 6 for Visit 7. The following is the procedure for Visit 7.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
24hr±15min
④ Sample collection for coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.3.5 Visit 8 (Day 8)

The subject shall be instructed and reminded to visit the hospital within 72hr±120min from the time of Visit 6 for Visit 8. The following is the procedure for Visit 8.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
After administration at Day 6, 72hr±120min
④ Sample collection for coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.3.6 Visit 9 (Day 10) – End of study

The subject shall be instructed and reminded to visit the hospital within 120hr±180min from the time of Visit 6 for Visit 9. The following is the procedure for Visit 9.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
After administration at Day 6, 120hr±180min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay
⑤ Termination of cohort and notification of post-study safety visit

5.2.4 Cohort 5

For Cohort 5, single IV administration of ISU304 75 IU/kg is performed and ISU304 150 IU/kg is subcutaneously administered within 30 min. (±5 min.) of IV administration. Afterwards, daily repeated subcutaneous administration (total 9 subcutaneous injection) for 8 days is performed with 24 hours interval. Based on the 1st subcutaneous administration, blood sampling for PK/PD analysis is performed for 312 hours. The details for each visit are as follows.

5.2.4.1 Screening Visit (Day -30 to 0)

The screening visit should be conducted within -30 days to the administration date. It should be scheduled in consideration of the fact that some tests for the inclusion/exclusion criteria will be conducted in an overseas central laboratory. The following is the procedure for the Screening Visit.

① Obtaining written informed consent from the subject
② Assigning screening no.
③ Collecting demographic data, hemophilia B status, medical and medication history (determining eligibility based on inclusion/exclusion criteria)
④ Physical examination, assessment of vital signs and electrocardiogram
⑤ Blood/urine sample collection for laboratory tests (only D-dimer test is conducted for blood coagulation, retest is allowed during screening period as judged by the Investigator), FIX inhibitor test

5.2.4.2 Visit 1 (Day 0) - Registration and Administration of Investigational Product

Visit 1 is for the 1st administration of investigational product (ISU304 75 IU/kg IV administration + ISU304 150 IU/kg subcutaneous administration) and the timing of the 1st administration is identical to the time that the subject will have to visit the hospital on subsequent visits. So, it is important to set the time of the 1st administration with consideration for the subject's schedule in the next 9 days. The investigator must observe the administration site for at least 30 minutes to 1 hour after the administration of investigational product for AEs. The following is the procedure for Visit 1.

① Assigning registration no. after final eligibility assessment
② Checking for additional medical history and concomitant medications
③ Physical examination, assessment of vital signs and electrocardiogram
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, Bethesda assay and blood sampling for PK/PD analysis before administration of investigational product
⑤ IV administration of ISU304 75 IU/kg is followed by subcutaneous administration of 150 IU/kg of ISU304 within 30 min. (±5 min.) of IV administration
⑥ Blood sampling for PK/PD analysis right after and 30 min. after IV administration of ISU304 75 IU/kg
⑦ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑧ Notification of visit schedule and scheduling of next visit

5.2.4.3 Visit 2 (Day 1) - Visit 9 (Day 8) - Repeated Administration of Investigational Product

The subject shall be instructed and reminded to visit the hospital in 24hr±15min intervals starting from the time of the 1st subcutanoues investigational product administration for Visits 2 to 9. The following is the procedure for Visits 2 to 9.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs
③ Blood sample collection for PK/PD analysis before administration of investigational product
④ Sample collection for coagulation test
⑤ Subcutaneous administration of 150 IU/kg of ISU304
⑥ Checking for AEs at least 30 min to 1 hr after investigational product administration
⑦ Notification of visit schedule and scheduling of next visit

5.2.4.4 Visit 10 (Day 9)

The subject shall be instructed and reminded to visit the hospital within 24hr±15min from the time of Visit 9 for Visit 10. The following is the procedure for Visit 10.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
After administration at Day 8, 24hr±15min
④ Sample collection for coagulation test
⑤ Notification of visit schedule and scheduling of next visit

5.2.4.5 Visit 11 (Day 11)

The subject shall be instructed and reminded to visit the hospital within 72hr±120min from the time of Visit 9 for Visit 11. The following is the procedure for Visit 11.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
After administration at Day 8, 72hr±120min
④ Sample collection for coagulation test

⑤ Notification of visit schedule and scheduling of next visit

5.2.4.6 Visit 12 (Day 13) – End of study

The subject shall be instructed and reminded to visit the hospital within 120hr±180min from the time of Visit 9 for Visit 12. The following is the procedure for Visit 9.

① Checking for AEs and concomitant medications
② Physical examination, assessment of vital signs and electrocardiogram
③ Blood sample collection for PK/PD analysis
After administration at Day 8, 120hr±180min
④ Blood/urine sample collection for laboratory tests, FIX inhibitor test, anti-drug antibody test, and Bethesda assay
⑤ Termination of cohort and notification of post-study safety visit

5.2.5 Unscheduled Visits (if needed)

Additional visit beyond the designated will be conducted upon request from a subject or subject's representative, or by the investigator's decision. The investigator shall confirm the reason for the unscheduled visit with the subject or the subject's representative.

The following items shall be collected upon additional visits according to the investigator's judgment of their necessity.

① Weight/height
② Checking for AEs and concomitant medications
③ Physical examination, assessment of vital signs
④ Blood/urine sample collection for laboratory tests

5.2.6 Post-Study Safety Visit

If possible, subjects will visit the hospital 2 weeks (+1 week) after end of a cohort (or premature termination) to collect blood, saliva or oral epithelial cell sample for FIX inhibitor test, anti-drug antibody test, and Bethesda assay. If the subject is difficult to visit, the consent form should be delivered to the subject in advance by mail/courier. The Investigator shall explain the revised (added) clinical study method by wire telephone or other means and obtain written consent from the subject.

After the written consent has been obtained from the subject, the Investigator shall visit the subject's home or workplace to collect the specimen as possible among the blood, saliva, or oral epithelium.

As the post-study safety visit is not a scheduled visit, it is not a protocol violation even if the subject does not carry out the post-study safety visit. However, when the result of anti-drug antibody and neutralized antibody test conducted at the post-study safety visit is positive, an additional visit may be conducted for a follow-up test to ensure the subject’s safety.

① Checking for AEs and concomitant medications
② Blood sample collection for FIX inhibitor test, anti-drug antibody test, and Bethesda assay

6 Subject Withdrawal or Termination

6.1 Reasons for Withdrawal or Termination

Subjects may withdraw the informed consent or discontinue their participation in the clinical study at any time without specifying the reasons. The investigator or sponsor is also able to discontinue the clinical study of a subject for safety or administrative reasons. A subject may withdraw from the study in any of the following cases.

- Withdrawal of consent by the subject or the subject's representative
- Major protocol violations*
- Dispensing/dosing error of the investigational products
- Occurrence of an AE that leads to treatment discontinuation
- Follow-up loss
- Investigator or sponsor's decision that continuation of the clinical study is not appropriate
- Development of FIX inhibitor (confirmed by sponsor)

* Major protocol violations are defined as below.

- Failure to obtain informed consent (i.e., no documentation in source data or informed consent form)
- Violation of the inclusion/exclusion criteria
- Use of prohibited medication

For minor protocol violations which are not deemed to influence the analysis of study results, the degree of violation or delay shall be exactly mentioned, and when the clinical study result report is written, the investigator, sponsor, monitor, and biostatistician shall comprehensively evaluate the analysis.

6.2 Handling of Subject Withdrawals or Termination

The principal investigator is responsible for conducting the clinical study in accordance with the protocol. Investigators should take necessary actions (e.g., written notice of the next visit, phone monitoring, etc.) to conduct the study as planned.

If the subject discontinues the study for any reason, the investigator must try to collect the planned data for the last visit (end of a cohort) from the subject. The data collected from the subject until the point of discontinuation can be used in the analysis. The investigator shall inform the subject of this when possible.

If the study of a subject is prematurely terminated for any reason, the investigator should promptly inform the subject or his representative, ask the reason for termination, and conduct appropriate therapy and follow-up for the subject if there is an unresolved AE.

7 Statistical Considerations

7.1 Endpoints

7.1.1 Basic Subject Information

- Demographic data, hemophilia B status, medical history, medication history

7.1.2 Safety Endpoints

- AEs after the administration of investigational products (local/systemic/other)
- Physical examination
- Vital signs
- Electrocardiogram
- Laboratory tests
- FIX inhibitors (or neutralizing antibodies) and anti-drug antibodies to investigational products

7.1.3 Pharmacokinetic/ Pharmacodynamic Endpoints

- PK endpoints: observed maximum plasma concentration (C_{max}), terminal phase elimination half-life ($t_{1/2}$), total plasma clearance (CL), volume of distribution at steady state (V_{ss}), area under curve (AUC; $AUC_{(0-t)}$ and $AUC_{(0-inf)}$), mean residence time (MRT), incremental recovery (K), time to maximum plasma concentration (T_{max}) for FIX activity and FIX product
- PD endpoint: activated partial thromboplastin time (aPTT)

7.2 Statistical Analysis

7.2.1 Analysis Population

Analysis sets are defined as below.

- Safety analysis set: All subjects who receive at least one dose of investigational products
- PK set: Subjects in the safety analysis set who complete all procedures planned for the cohort and for whom PK samples have been obtained from at least 4 post-dose points; the 4 post-dose points should include at least 2 points within 3 hours after IV administration, or within 12 hours after SC administration. However, Cohort 4 and Cohort 5subjects can be included in the PK set with

samples from 48 hours after the 1st administration

7.2.2 Analysis of Basic Subject Information

For each cohort, descriptive statistics will be given for each endpoint for each subject (i.e., mean (± standard deviation), median, minimum, and maximum for continuous data; frequency and percentage for categorical data) using SAS software (SAS Institute Inc., Cary, USA).

7.2.3 Safety Analysis

7.2.3.1 Adverse Events (AEs)

The incidence of reported AEs (local/systemic/other) after investigational product administration shall be calculated by cohort, with the relevant 95% confidence intervals and number of events being estimated.

The AEs shall be categorized according to severity, causal relationship with the investigational products, and the number of occurrences and percentage shall be calculated. The numbers of AEs, ADRs, and SAEs as well as the numbers of subjects experiencing them shall be calculated to estimate incidence.

Additional analysis shall be conducted for AESIs using identical methods applied to AEs.

All AEs shall be analyzed according to system organ class (SOC) and preferred term (PT) using the latest version of the MedDRA.

7.2.3.2 Physical Examination

Normal/abnormal changes in physical examination results before and after administration of the investigational products shall be summarized in terms of frequency and percentage by cohort.

7.2.3.3 Vital Signs

Descriptive statistics for vital signs measured before and after administration of the investigational products shall be presented in mean (± standard deviation), median, minimum, and maximum by cohort.

7.2.3.4 Electrocardiogram

Descriptive statistics from electrocardiograms conducted before and after administration of the investigational products shall be presented in mean (± standard deviation), median, minimum, and maximum by cohort. Normal/abnormal changes shall be summarized in terms of frequency and percentage.

7.2.3.5 Laboratory Tests

Descriptive statistics from laboratory tests before and after administration of the investigational products shall be presented in mean (± standard deviation), median, minimum, and maximum by cohort. Normal/abnormal changes shall be summarized in terms of frequency and percentage.

7.2.3.6 FIX Inhibitors and Anti-drug Antibodies to the Investigational Products

The analysis of FIX inhibitors (neutralizing antibodies) and anti-drug antibodies shall be conducted for each subject by cohort at the designated central laboratory (HTI). A separate Bethesda assay shall be carried out at a different central laboratory (SCL). Changes in neutralizing antibodies and anti-drug antibodies before and after administration of the investigational products shall be summarized in terms of frequency, percentage, and titer.

7.2.4 Pharmacokinetic/ Pharmacodynamic Analysis

PK/PD analysis by cohort shall be conducted for PK set at the designated central laboratory (HTI). The descriptive statistics of PK/PD endpoints shall be presented in mean (± standard deviation), median, minimum, and maximum.

PK/PD analysis will be conducted via standard non-compartmental methods using WinNonlin software (Pharsight, CA, USA). NONMEM software (ICON, MD, USA) will be used for additional analysis using the compartmental method, if applicable. In this case, data from subjects with missing values will be included. The bioavailability of ISU304 in subcutaneous administration will also be calculated.

7.2.5 Handling of Missing Values

Missing values will not be replaced.

8 Adverse Events (AEs)

8.1 Definition of Terms

- Adverse event (AE)

An AE is any untoward or unfavorable medical occurrence in a human study subject, including any abnormal sign (e.g., an abnormal laboratory finding), symptom, or disease, temporally associated with the use of an investigational product, whether or not considered related to the investigational product.

- Adverse drug reaction (ADR)

In the pre-approval clinical experience with a new investigational product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to an investigational product related to any dose (i.e. the relationship cannot be ruled out) should be considered ADRs.

- Unexpected ADR

An ADR, the nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

- Serious AE and ADR (SAE and SADR)

AEs or ADRs occurring at any dose of an investigational product that fall into any one of the following cases.

  - Occurrence of death or life threatening risk
  - Requires or prolongs hospitalization
  - Causing severe or permanent disabilities/impairment of functions
  - Occurrence of congenital defects or anomalies in fetus
  - Occurrence of other medically significant events such as drug dependency or abuse, blood disease, etc.

8.2 Collection and Documentation of Adverse Events

AEs shall be collected during the clinical study period, from the time of investigational product administration to end of a cohort. Any medical incidences before the administration of the investigational product shall be recorded as current medical history.

AEs shall be reported with details on onset/end date, severity, outcome, action taken to investigational products, causal relationship with investigational products, causal relationship with other drugs, treatment given for AE, and seriousness. When documenting AEs, the investigators should record the overall diagnosis or symptoms using standard medical terms (according to the latest version of the MedDRA).

AEs occurring during the study period shall be followed up until recovery (or stabilization), or until the case is lost to follow up. AEs occurring after the study completion should be reported only when they are serious and causally related to investigational products.

8.3 Assessment of Adverse Events

8.3.1 Severity

The severity of an AE will be determined according to following criteria.

- Mild: Tolerable AEs
  (Although signs and symptoms are recognizable, they are easily tolerated, with mild discomfort not causing a loss of time in daily activities; the symptoms do not require treatment or medical assessment, the signs and symptoms are temporary.)
- Moderate: AEs presenting considerable inconvenience to activities of daily living*
  (AEs cause a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually improved by simple therapeutic measures; may cause some interference to functions.)
- Severe: AEs that make activities of daily living* impossible to conduct
  (AEs interrupt the subject's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.)

* Activities of daily living: all activities that occur in day-to-day life to maintain one's own physical well-being (e.g., Bathing, dressing, eating, taking medications, hygiene maintenance, personal care)

8.3.2 Causal Relationship with Investigational Product

The investigator evaluates all collected AEs based on temporal relationship and his/her clinical judgment. Causal relationships are assessed using the following categories.

- Definitely related

There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The temporal order of the investigational product administration and AE incidence is reasonable and the AE cannot be explained by concomitant diseases, other medications, or chemical substances. The AE disappears when the drug is discontinued. Symptoms upon rechallenge (when possible) must be pharmacologically or phenomenologically definitive.

- Probably related

There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The temporal order of the investigational product administration and AE incidence is reasonable and the possibility of the AE being caused by concomitant diseases, other medications, or chemical substances is low. The AE disappears when the drug is discontinued. Information of rechallenge is not necessary.

- Possibly related

There is some evidence to suggest a causal relationship (e.g., the temporal order of the investigational product administration and AE incidence is reasonable). However, other factors may have contributed to the AE (e.g., the subject's clinical condition, other concomitant events). Although an AE may only be evaluated as "possibly related" soon after occurrence, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related".

- Probably not related

AE is deemed to be not causally related to the administration of the investigational product due to their temporal relationship (e.g., temporal order between administration and AE occurrence does not support causality), the AE is reasonably explained by concomitant diseases, other medications, or chemical substances (e.g., the subject's clinical condition, other concomitant treatments).

- Definitely not related

AE is clearly unrelated to the administration of the investigational product and there is clear evidence of a different causal factor. There must be an alternative, and definitive etiology documented by the clinician.

- Unknown

Information is insufficient or contradictory to such an extent that a judgment cannot be made and it is not possible to supplement or verify such information.

8.4 Adverse Event Reporting

8.4.1 SAE Reporting

The principal investigator shall report all SAEs to the sponsor during the clinical study within 24 hours from the time known to the investigator by phone/fax, regardless of causal relationship with the investigational products. SAEs occurring within 14 days after the completion of the study shall be reported by the same process. The investigator must use the subject identification code when reporting SAEs.

Contact information for safety reporting to ISU ABXIS:

PV staff			
E-mail	: safety.isuabxis@isu.co.kr	Phone/Fax	: 070-4665-4308 / 031-696-4771
Address	: 5F, Global R&D Center Building C, 712-gil 22, Daewangpangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do		

Minimum requirements for initial SAE report are as follows.

- Subject information
- Suspected drug and causal relationship with investigational products
- Reporting person information
- Name of SAE

Investigators must conduct follow up reporting when additional information is obtained regarding reported SAEs. The investigator shall report periodically until the SAE is resolved (e.g., recovery or follow-up becomes impossible).

8.4.2 SUSAR Reporting

When a Suspected Unexpected Serious Adverse Reaction (SUSAR) occurs, the principal investigator must report it to the IRB to determine whether the study should be continued or not, and help the sponsor to report the SUSAR immediately to the Ministry of Food and Drug Safety (MFDS).

- Fatal or life threatening SUSARs must be reported immediately. Sponsor has to report them to the MFDS by phone, fax, or documentation, as soon as possible, within 7 days of initial knowledge,

and submit a full report within 15 days of initial knowledge if possible.

- All other SUSARs have to be reported as soon as possible, within 15 days following initial reporting to or knowledge by sponsor.

During the study period, the investigator shall ensure subject safety, and take prompt and proper measures to minimize AEs. The duties of the respective persons-in-charge upon the incidence of SUSARs are as follows.

- Principal investigators: immediately report the SUSAR to the IRB of each center and sponsor, and discontinue the clinical study in part or entirety until further instruction.
- Sub-investigators: immediately report the SUSAR to the principal investigator and sponsor.
- IRB: take measures necessary to the principal investigator (e.g., discontinuation of clinical study in part or entirety)
- Sponsor: upon receiving a SUSAR report from the principal investigators or sub-investigators, immediately submit a copy of the report from the principal investigator or sub-investigator to the MFDS and swiftly inform the study centers

Other AEs not defined above will be tracked until 14 days after the last investigational product administration.

8.5 Pregnancy Reporting

Pregnancy itself during the clinical study will not be regarded as an AE. Selective abortion without complications (not including therapeutic abortion) and hospitalization for the normal delivery of a healthy child are also not considered as AEs.

However, if the subject's spouse or female partner becomes pregnant during the study period (within 30 days from the administration of investigational product), the subject must be immediately dropped out of the study. The investigator shall submit the pregnancy report to the sponsor within 24 hours after the investigator is made aware of pregnancy. Even if the subject withdraws or terminates the study, the investigator shall follow-up the progress of the pregnant woman and her fetus until delivery.

If the pregnant woman experiences a serious complication, natural abortion, extra uterine pregnancy, stillbirth, neonatal death, congenital malformation, etc., these shall be deemed as SAEs, and the investigator shall report them.

9 Ethical, Legal, and Administrative Considerations

9.1 Study Centers

The directors of the study centers shall provide the locations, facilities, and professionals to enable the appropriate execution of the study.

9.2 Institutional Review Boards

When obtaining approval for the protocol or when modifying an approved protocol, the protocol or modified protocol has to be approved by the IRB and the MFDS (if necessary). No subject is allowed to participate in the study prior to approval.

9.3 Subject Information Sheet and Informed Consent Form

Prior to the execution of all study related procedures, the investigator shall fully explain the study to each subject (or the subject's representative), and obtain written consent from the subject.

The investigator shall take enough time to thoroughly assess the eligibility of each subject through interviews and/or tests.

The investigator shall keep the signed original of the informed consent form in the investigator's file, and provide copies of the signed informed consent form and subject information sheet to the subject (or representative). If the subject information sheet and informed consent form are modified, they shall be re-approved by the IRB, as well as the ongoing subject (or representative).

Refer to [Attachment 2. Subject Information Sheet and Informed Consent Form]

9.4 Adherence to the Protocol

The investigator will ensure that the clinical study is conducted in compliance with the Declaration of Helsinki to protect the subject's rights and welfare. The investigator shall be familiar with the Korea Good Clinical Practice (KGCP) and the protocol, and respond actively to subject safety issues.

9.5 Post-study Treatment for Subjects

The investigator shall ensure that a subject who drops out of the study or does not respond to the drug can receive other forms of appropriate treatment.

9.6 Subject Indemnification

The sponsor shall compensate the subject in the case of damages caused by AEs of the investigational product or in the treatment of AEs, where the investigational product is the direct cause of the damage according to Attachment 3. Subject Indemnification.

Refer to [Attachment 3. Subject Indemnification]

9.7 Monitoring

To protect subject's rights and welfare, confirm the accuracy, integrity, and verifiability of data through comparison between reported study related data and source documents, and check if the clinical study has been performed in compliance with the approved protocol and KGCP, the study centers shall be monitored.

The clinical research associate (CRA) from DreamCIS Inc. shall monitor the study, evaluate the study progress, and check whether the investigator's duties are being fulfilled as per the protocol and regulations through routine site visits and phone calls. When visiting the center, the CRA shall check whether the originals of subject's records, CRFs, drug management records, and study-related data (e.g., investigator's files) are being kept, and discuss with the investigator upon finding inconsistencies or problems in the clinical study records.

9.8 Storage of Clinical Study Data

The investigator shall keep the data and records related to the execution of the study in a safe place, maintain their security, and store them for 3 years from the date of product approval in the lastly approved country. After the clinical study report has been completed, documents related to the study shall be transferred to the data storage manager. If the investigator wishes to discard or to move study related records to another place, the investigator shall notify the sponsor in advance.

9.9 Data Safety Monitoring Board and Data Monitoring Committee

An independent Data Safety Monitoring Board (DSMB) and Data Monitoring Committee (DMC) shall perform interim assessments of PK/PD and safety data at the end of each cohort. The DMC assesses the PK/PD data while the DSMB accesses the safety data. The DSMB is comprised of members independent to the study whom include 1 clinician with expertise regarding the clinical aspects of hemophilia (DSMB Chair), 1 clinical pharmacologist, and 1 biostatistician. The DMC is comprised of the investigators of this study, the sponsor, PK/PD experts, and etc.

Upon the termination of each cohort (after the last visit of the final subject), the PK/PD and safety data are analyzed before decisions are made regarding the commencement of the next cohort, target dose, and blood collection period. In the presence of subject safety concerns, the study may be put on hold or terminated, and decisions regarding the necessity of additional subjects as well as subject/cohort replacement/suspension may also be made. Ad hoc specialists may be invited to participate as non-voting members of DSMB at any time if additional expertise is needed.

9.10 Audit and Inspection

To ensure that the KGCP and all related regulations are observed, the sponsor or a person authorized by the sponsor may perform audits while the MFDS may conduct inspections of the study. After the relevant notice is given to the investigator, the investigator shall respond to audit or inspection requests, allow the auditor or inspector direct access to all clinical study related documents, and consent to allocating sufficient time to fully discuss all observations and related issues.

9.11 Confidentiality

All clinical study results and documents shall be kept confidential. The investigator, contract research organization, and staff from the sponsor shall not expose any study related information without the sponsor’s signed approval. Records that may be used to identify the subject shall be kept confidential, and all study related documents will be recorded with the screening number instead of the subject’s name. Even if the results of the clinical study are published, the subjects’ identities are to be kept confidential.

The investigator shall be aware of the fact that with the conclusion of the clinical study contract, the sponsor, monitor, or auditor may review or copy relevant documents to verify the subjects’ medical

records and CRF records. Such information shall be kept confidential, and facilities for confidential record keeping shall be established with appropriate management criteria.

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11 Appendix

Appendix 1. List of Study Centers and Principal Investigators

Appendix 2. Subject Information Sheet and Informed Consent Form

Appendix 3. Subject Indemnification

Appendix 4. Table – Assessment of Local Adverse Events

Appendix 5. Table – Assessment of Systemic Adverse Events

Statistical Analysis Plan

Protocol Identification No.	ISU304-001
Title	A Phase 1, Open-label, Multi-center, Dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of ISU304 in Previously Treated Hemophilia B Patients
Trial Phase	Phase 1
Protocol Version	11-SEP-2018/Version 5.3
Sponsor	ISU ABXIS
Author	Kyung-Jin You
Date and Version	19-MAR-2019/Version 3.0

Effective Date: 2018-10-12

Signature Page

A Phase 1, Open-label, Multi-center, Dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of ISU304 in Previously Treated Hemophilia B Patients

Authored by:

Name / Title & Affiliation	Signature	Date
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Approved by:

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History of Revisions

Version	Date of Approval	Author Name
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V2.0	02-JAN-2019	Kyung-Jin You
V3.0	19-MAR-2019	Kyung-Jin You

Table of Contents

Abbreviations

Abbreviations	Definition
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
CS	Clinical Significance
FIX	Factor IX
HIV	Human Immunodeficiency Virus
IP	Investigational Products
IU	International Unit
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Non Clinical Significance
PT	Preferred Term
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Class
TLFs	Tables, Listings, Figures
WHODDE	World Health Organization Drug Dictionary Enhanced

1. Purpose of the Statistical Analysis Plan

The purpose of this document is documentation for the details for analyzing the data collected in the ISU304-001 study.

This document is based on the statistical analysis method described in protocol, and results of the analysis in this document will be included in the clinical study report.

If any additional analysis undescribed in this document is required, it will be written in separate document (ex. Additional Analysis Plan).

2. Study Features

Study title	A Phase 1, Open-label, Multi-center, Dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of ISU304 in Previously Treated Hemophilia B Patients
Study duration	Approximately 24 months after the protocol approval from the institutional review boards (IRBs)
Indication	Previously treated hemophilia B patients
Study objective	To investigate the safety, pharmacokinetics and pharmacodynamics of ISU304 (recombinant factor IX with increased activity) in previously treated hemophilia B patients
Phase and design	Phase 1, open-label, multi-center, dose-escalation
Investigational products	• Study drug: ISU304 75, 150IU/kg, intravenous or subcutaneous administration • Active control: BeneFIX 75 IU/kg, intravenous administration • Rescue drug: Treatment currently used by subject to treat hemophilia B

Administration and follow-up period	• Cohort 1 (n=3): 7 days Single intravenous administration of BeneFIX (75 IU/kg) with 72 hours of observation, followed by single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation • Cohort 2 (n=3): 7 days Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (75 IU/kg) with 72 hours of observation • Cohort 3 (n=3): 9 days Single intravenous administration of ISU304 (75 IU/kg) with 72 hours of observation, followed by single subcutaneous administration of ISU304 (150 IU/kg) with 120 hours of observation • Cohort 4 (n=5): 10 days Single subcutaneous administration of ISU304 (150 IU/kg) per day for 6 days with 240 hours of observation • Cohort 5 (n=minimum 3, maximum 5): 15 days Single intravenous administration of ISU304 (75 IU/kg) is followed by subcutaneous ISU304 (150 IU/kg) administration for 9 days with 312 hours of observation
Planned number of subjects	At least 12 subjects (including duplication)
Inclusion criteria	1) Previously treated male patients with moderate or severe hemophilia B (documented FIX activity ≤ 2% and exposed to any FIX product for ≥ 150 exposure days (estimated)) 2) Patients must be 12 to 65 years old at the time of screening 3) Patients who have discontinued a previously treated FIX product at least 4 days prior to the administration of investigational product 4) HIV negative, or if HIV positive with a CD4 count > 200/μL (documented < 200 particles/μL or ≤ 400,000 copies/mL) at the time of screening 5) Voluntary consent to participate in the study
Exclusion criteria	1) Patients with a history or a family history of FIX inhibitors 2) Patients with FIX inhibitors (positive result for BeneFIX or ISU304 from inhibitor tests) at the time of screening 3) Patients who have a history of thromboembolic events (myocardial infarction, cerebrovascular disease, venous thrombosis, etc.) 4) Patients with known hypersensitivity, allergy, or anaphylaxis to any FIX

	product or hamster protein 5) Patients receiving treatment with a FIX product or a bypass agent within 4 half-lives for the agent used (at least 96 hours) prior to the administration of the investigational product 6) Patients who have been exposed to long-term administration(exceeding 14 days) of immunomodulating agents or immunosuppressants such as α-INF or adrenocortical hormones over the past 3 months or who are currently receiving or planning to receive such treatment during the study period 7) Patients who have been administered vaccines during the period of 6 months prior to the administration of the investigational product or plan to receive vaccines during the study period 8) Patients with any other co-existing bleeding disorder (Von Willebrand disease, etc.) 9) Patients with positive D-dimer results (≥ 0.5 μg/mL) at the time of screening 10) Patients with platelet counts less than 100,000/μL at the time of screening 11) Patients with ALT, AST levels 5 times greater than upper normal limit or total bilirubin, serum creatinine levels 2 times greater than upper normal limit at the time of screening 12) Active hepatitis patients who are clinically significant HBs Ag positive or anti-HCV Ab positive at the time of screening 13) Patients scheduled for surgery during the study period 14) Patients participated in another study within 30 days before screening or scheduled to participate in any other study during the study period
Safety endpoints	• Adverse events after the administration of investigational products (local/systemic/other) • Physical examination • Vital signs • Electrocardiogram • Laboratory tests • FIX inhibitors (or neutralizing antibodies) and anti-drug antibodies to investigational products
Other endpoints	Demographic data, hemophilia B status, medical history, medication history

Analysis method	• Analysis population - Safety analysis set: All subjects who receive at least one dose of investigational products - PK set: Subjects in the safety analysis set who complete all procedures planned for the cohort and for whom PK samples have been obtained at least 4 times after administration. • PK Analysis: Using WinNonlin software (Pharsight, CA, USA) • Bioavailability analysis for subcutaneous administration • Other statistical analysis - For each cohort, descriptive statistics will be given for each endpoint for each subject using SAS software (SAS Institute Inc, Cary, USA)

3. Sample Size Calculation

At least 12 subjects who meet all of the inclusion criteria and none of the exclusion criteria including duplication will be enrolled.

4. Sequence of Planned Analyses

4.1 Interim Analysis

Interim analysis is not planned in this study.

4.2 Final Analysis

The statistical analysis of this study will be performed after all data is collected and database lock is completed.

5. Changes to the Planned Analyses in the Protocol

The PK/PD analysis is described in Protocol but PK/PD analysis will be conducted at the designated central laboratory (HTI). So this document does not include this analysis.

6. Analysis Sets

Safety Analysis Set

All subjects who receive at least one dose of investigational products

7. Protocol Deviations

The additional protocol deviations other than analysis set criteria described in '6. Analysis Sets' are not considered.

8. General Specifications for Statistical Analyses

8.1 Analysis Software

All statistical analysis will be carried out with SAS Software (SAS Institute Inc, Cary, USA) version 9.4 or more recent version.

8.2 Multiple Comparison and Multiplicity

The consideration of multiple comparison and multiplicity is not necessary in this study.

8.3 Planned Subgroup

Subgroup analysis is not planned in this study.

8.4 Planned Covariate

Covariate analysis is not planned in this study.

8.5 Summary Statistics

For the continuous data, descriptive statistics such as mean, standard deviation, 25th percentile, median, 75th percentile, minimum and maximum value shall be obtained. For the categorical data,

descriptive statistics such as frequency and percentage shall be provided.

8.6 Methods for Missing Data and Incomplete Data

Missing data and incomplete data will not be replaced.
Exceptionally in laboratory test, if results include incomplete data, it will be converted to numeric value and included in the analysis as following example. Also incomplete data will be listed.

- if data including '<number', it will be replaced 'number - 0.001' (e.g. <2 → 1.999).
- if data including '>number', it will be replaced 'number + 0.001' (e.g. >100 → 100.001).

8.7 Other Statistical Considerations

The analysis will be carried out according to considerations as follows. If additional considerations on each analysis item is required, the corresponding options will be described in '10.1 Derivation'.

- In Cohort 1, Cohort 2, Cohort 3, 'Baseline' is '1st Hospitalization (Before 1st injection)' visit. And in Cohort 4 and Cohort 5, 'Baseline' is 'Before 1st injection' visit.
- The criteria for converting day to month are as follows: 1month=30days
- When calculating percentage for categorical variables except for 'Analysis Sets' summary, the denominator is the number of subjects in each cohort. Only in case of analysis by type of Medical History/Surgical History/Prior Medication/Concomitant Medication, the denominator the number of subjects having Medical History/Surgical History/Prior Medication/Concomitant Medication in each cohort.
- The calculating criteria of percentage for 'Analysis Sets' summary will be described in 9.1 Disposition of Subjects'.
- The 95% confidence interval will be estimated by using exact method.

8.8 Reporting Conventions

- The descriptive statistics (mean, standard deviation, 25th percentile, median, 75th percentile, minimum, maximum, percentage) rounds to 2 decimal places.
- If the calculated percentage is less than 0.01, it will be presented as '<0.01'.

9. Evaluation of Study Data

All analysis will be applied for Safety Analysis Set as follows.

Analyses	Safety Analysis Set
Demographics	✓
Medical History	✓
Prior and Concomitant Medications	✓
Safety Evaluation	✓

9.1 Disposition of Subjects

The following 'Patient disposition' and 'Analysis Sets' will be presented as a figure.

Patient disposition

- The number of screening subjects
- The number of screening failure subjects and reason for screening Failure
- The number of enrolled subjects
- The number of early withdrawal subjects and reasons for early withdrawal
- The number of study completion subjects

Analysis Sets

- The number of enrolled subjects
- The number of Safety Analysis Set of enrolled subjects
- The number of subjects excluded from Safety Analysis Set of enrolled subjects and the criterion of excluded from Safety Analysis Set

Additionally for 'Analysis Sets', the table including the frequency and percentage will be presented.

The following subjects will be listed.

- Listing of Subjects with early withdrawal
- Listing of Subjects enrolled in multiple cohorts

9.2 Demographics

For following continuous variable, the descriptive statistics for each cohort will be presented.

- Age
- Height
- Weight

For following categorical variables, the descriptive statistics for each cohort will be presented.

- Gender
- Age

The above demographics for each subjects will be listed.

9.3 Medical History

For following continuous variable, the descriptive statistics for each cohort will be presented.

- Duration of Hemophilia B

For following categorical variables, the descriptive statistics for each cohort will be presented.

- Duration of Hemophilia B
- Family History of Hemophilia B
- History of FIX Inhibitor
- Family History of FIX Inhibitor
- FIX activity ≤ 2% before screening visit
- FIX product 150 exposure days at the time of screening
- Medical History[a]
- Surgical History[b]

[a] Medical History: Any past or concomitant disease at screening (last 3months)

[b] Surgical History: Any surgical history from prior 3 months ago to screening visit

The medical history and surgical history will be classified as SOC under the classification standard of recent version MedDRA. If one subject has 2 or more medical history/surgical history in one category (SOC), it will be analyzed as one in the category. Also, the same subject may appear in

different category in duplicate.

9.4 Prior and Concomitant Medications

For following categorical variables, the descriptive statistics for each cohort will be presented.

- Prior Medication[a]
- Concomitant Medication[b]

[a] Prior Medication: any drugs started from 3 months before screening visit to 1st injection

[b] Concomitant Medication: any drugs started after 1st injection

The prior and concomitant medication will be classified as Level 1, Level 2 under the classification standard of recent version WHODDE. If one subject taken 2 or more medication in one category (Level 1, Level 2), it will be analyzed as one in the category. Also, the same subject may appear in different category in duplicate.

9.5 Safety Evaluation

9.5.1 Adverse Events

The summary table for the following AE will be presented by each cohort. In the summary table, the number and proportion of subjects to whom following AE occurred, and its 95% confidence interval and the number of following AEs will be estimated.

- Adverse Event
- Adverse Drug Reaction[a]
- Serious Adverse Event
- Serious Adverse Drug Reaction[a]
- Early Withdrawal due to AE[b]
- Adverse Event of Special Interest[c]

[a] Adverse Drug Reaction: AE including whose 'Causal Relationship with IP' is assessed "Definitely related", "Probably related", "Possibly related", "Unknown"

[b] Early Withdrawal due to AE: AE whose 'Reason for discontinuation' of 'Study Conclusion' is

'Occurrence of an AE that leads treatment discontinuation' and 'Action taken to IP' is "Interrupted"
[c] Adverse Event of Special Interest: It is Thrombosis/embolism, Spontaneous bleeding, Inhibitor generation/seroconversion, Anaphylaxis and collected in other AE only

Additionally, the summary table for the following local[1]/systemic[2]/other[3] AE will be presented by each cohort. In the summary table, the number and proportion of subjects to whom local/systemic/other AE occurred, and its 95% confidence interval and the number of following AEs will be estimated.

- Adverse Event
- Adverse Drug Reaction
- Serious Adverse Event
- Serious Adverse Drug Reaction
- Early Withdrawal due to AE

[1] Local AE: AEs occurring within 1 hour after the administration of investigational products including pain, tenderness, erythema/redness, urticaria/pruritus, and induration/swelling

[2] Systemic Adverse Event: AEs occurring within 1 hour after the administration of investigational products including fever, fatigue/malaise, chill/pyrexia, , headache, myalgia, arthritis(arthralgia), decreased appetite/loss of appetite, diarrhea, vomiting, constipation, abdominal pain, mucosal skin reaction/rash, vasovagal syncope, dizziness, cough, acute bronchospasm, dyspnea, and hypersensitivity

[3] Other Adverse Event: AEs other than local or systemic AEs occurring after the administration of investigational products

For following item, the number and proportion of subjects with following local AE and systemic AE according to reported term, other AE according to the SOC, PT and its 95% confidence interval and the number of following local AE and systemic AE according to reported term, other AE according to the SOC, PT will be estimated. If one subject has 2 or more AE in one category, it will be analyzed as one in the category. Also, the same subject may appear in different category in duplicate.

- Adverse Event
- Adverse Drug Reaction
- Serious Adverse Event
- Serious Adverse Drug Reaction
- Adverse Event of Special Interest

For AE, local/systemic/other AE, AESI, the number of AEs and its percentage (%) by each cohort

according to following AE item will be presented.

- Seriousness
- Severity
- Outcome
- Action Taken to IP
- Causal Relationship with IP
- Causal Relationship with others
- Treatment Given for AE

The following AEs will be listed.

- Local Adverse Drug Reaction
- Systemic Adverse Drug Reaction
- Other Adverse Drug Reaction
- Local Serious Adverse Event
- Systemic Serious Adverse Event
- Other Serious Adverse Event
- Adverse Event of Special Interest
- Adverse Event of subjects whose Early Withdrawal due to Adverse Event
- Other Adverse Event whose reported term is 'Nab positive' or 'ADA positive'
- Other Adverse Event whose PT term include 'injection site'
- Adverse Event (each patient)

9.5.2 Laboratory Tests

For laboratory test, the descriptive statistics for each cohort will be presented in collected visit. And the change of baseline and end of cohort will be presented.
Additionally, the frequency and percentage of 'Normal/NCS', 'CS' in CD4 count of hematology test at screening will be presented. And the frequency and percentage of 'Normal', 'NCS', 'CS' in serology test at screening will be presented.

Also, 'Normal/NCS', 'CS' changes from baseline to end of cohort in hematology test(except for CD4 count), blood chemistry test, urinalysis and blood coagulation test will be summarized in terms of frequency and percentage by cohort.

9.5.3 Physical Examination

'Normal/NCS', 'CS' changes from baseline to end of cohort in physical examination results will be summarized in terms of frequency and percentage by cohort. The subjects with clinically significant results in physical examination will be listed.

9.5.4 Vital Signs

For vital signs results, the descriptive statistics for each cohort will be presented in collected visits. And the change of baseline and end of cohort will be presented.

9.5.5 Electrocardiogram

For each electrocardiogram results, the descriptive statistics for each cohort will be presented at each collected visit. And the change of baseline and end of cohort will be presented.
'Normal/NCS', 'CS' changes from baseline to end of cohort in electrocardiogram results will be summarized in terms of frequency and percentage by cohort.

9.5.6 FIX Inhibitor and Anti-drug Antibody Test

For FIX Inhibitor test and Anti-drug Antibody test, 'Positive', 'Negative' changes from 'Before injection' to 'After injection' in each results will be summarized in terms of frequency and percentage by cohort. If 'Positive' is confirmed once after injection, the subjects will be included 'Positive'.

In FIX Inhibitor test, if data is collected as 'LOQ', it will be analyzed as 'Negative', and if data is collected any numeric value, it will be analyzed as 'Positive'.
In Anti-drug Antibody test, data is collected as 'Positive', 'Negative'.

Additionally all FIX Inhibitor test and Anti-drug Antibody test results will be listed.

10. Derivation and Categorization for Variable

10.1 Derivation

The calculation formulas are as follow. If statistical analysis requires additional calculation formula, SAP may not be revised but the calculation formula will be specified in TLFs.

Age

- In case the date of Informed Consent month/day < Date of Birth month/day:
  Age = (Date of Informed Consent) - (Date of Birth) - 1
- In case the date of Informed Consent month/day ≥ Date of Birth month/day:
  Age = (Date of Informed Consent) - (Date of Birth)

Duration of Hemophilia B

- Year of 1st injection - Year of Diagnosis of Hemophilia B

Prior Medication

- Prior & Concomitant Medication Start Date < Date of 1st injection
- Prior & Concomitant Medication End date < Date of 1st injection
- Prior & Concomitant Medication Start Date is missing or unknown
- The following example will be included in Prior Medication.

Prior & Concomitant Medication Start Date	Prior & Concomitant Medication End date	Date of 1st injection
2015-10-06	Ongoing	2015-10-15
2015-10-UK	2015-10-10	2015-10-15
2015-10-UK	Ongoing	2015-10-15
2015-10-UK	2015-10-UK	2015-10-15
UK	2015-10-20	2015-10-15
UK	UK	2015-10-15

Concomitant Medication

- (Prior & Concomitant Medication Start Date < Date of 1st injection) & (Prior & Concomitant Medication End Date ≥ Date of 1st injection)

- (Prior & Concomitant Medication Start Date < Date of 1st injection) & (Prior & Concomitant Medication is 'Ongoing')
- Prior & Concomitant Medication Start Date ≥ Date of 1st injection
- In case Prior & Concomitant Medication End Date is unknown, it is not defined Concomitant Medication.
- The following example will be included in Concomitant Medication.

Prior & Concomitant Medication Start Date	Prior & Concomitant Medication End date	Date of 1st injection
2015-10-20	2015-10-30	2015-10-15
2015-10-06	Ongoing	2015-10-15
2015-11-UK	2015-11-UK	2015-10-15
2016-UK-UK	2016-UK-UK	2015-10-15
2015-10-UK	2015-10-20	2015-10-15
2015-10-UK	2015-11-UK	2015-10-15
2015-UK-UK	2016-UK-UK	2015-10-15

10.2 Categorization

Categories for each item are as follows.
The following defined category for continuous variable can be changed according to the distribution of actual collected data.

Item	Category
• Gender	Male
• Age	12 years ~ 19 years, 20 years ~ 29 years, 30 years ~ 39 years, 40 years ~ 49 years, 50 years ~ 59 years, 60 years ~ 65 years
• Duration of Hemophilia B	< Median, ≥ Median (Median: median for Duration of Hemophilia B in Safety Analysis Set)
• Family History of	Yes, No

Item	Category
Hemophilia B	
• History of FIX Inhibitor	No
• Family History of FIX Inhibitor	No
• FIX activity ≤ 2% before screening visit	Yes
• FIX product 150 exposure days at the time of screening	Yes
• Medical History	Yes, No
• Surgical History	Yes, No
• Prior Medication	Yes, No
• Concomitant Medication	Yes, No
• AE Seriousness	Yes, No
• AE Severity	Mild, Moderate, Severe
• AE Outcome	Recovered without Sequelae, Recovered with Sequelae, Continuing, Death, Unknown
• Action Taken to IP	Not Applicable, Interrupted
• Causal Relationship with IP	Definitely related, Probably related, Possibly related, Probably not related, Definitely not related, Unknown
• Causal Relationship with others	Concomitant Disease, Concomitant Medication, Other, Unknown, None

Item	Category
• Treatment Given for AE	None, Drug Therapy, Non-Drug Therapy, Drug, Non-Drug Therapy
• Laboratory Tests(Hematology test, Blood Chemistry test, Urinalysis, Blood coagulation test)	Normal/NCS, CS
• Laboratory Tests(Serology test)	Normal, NCS, CS
• Physical Examination	Normal/NCS, CS
• Electrocardiogram	Normal/NCS, CS

Appendix

- Mock up TLFs