Title Page

A PHASE 1/2, OPEN-LABEL, ADAPTIVE, DOSE-RANGING STUDY TO ASSESS THE SAFETY AND TOLERABILITY OF SB-525 (PF-07055480) (RECOMBINANT AAV2/6 HUMAN FACTOR 8 GENE THERAPY) IN ADULT SUBJECTS WITH SEVERE HEMOPHILIA A

Compound: SB-525 (Pfizer reference: PF-07055480)

Regulatory Agency Identifier Number(s)

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Amendment Number: 5

Phase: Phase 1/Phase 2

Short Title: Phase 1/2 Dose-Ranging Study of Recombinant AAV2/6 Human Factor VIII Gene Therapy SB-525 (Pfizer reference: PF-07055480) in Subjects with Severe Hemophilia A.

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Amendment 5	30-Mar-2023
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Document History

This amendment incorporates all revisions to date, including amendments made at the request of country health authorities and IRBs/ECs and any protocol administrative change letter(s).

Protocol Amendment Summary of Changes Table

Amendment 5 (30 March 2023)

Overall Rationale for the Amendment: change related to an addition of an optional liver biopsy substudy.

Section # and Name	Description of Change	Brief Rationale	Substantial or Nonsubstantial
Section 1.3 Schedule of Activities **Section 3. Objectives, Estimands, and Endpoints** **Section 8.2.7. Liver Assessment**	Addition of an optional liver biopsy substudy (and recommended related assessments).	The objective of this exploratory substudy is to assess the vector integration in the liver, to examine the liver tissue for any alteration and to provide a better understanding of the mechanism of action of the study drug in the liver.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial or Nonsubstantial
Section 8.2.14. Optional Liver Biopsy **Section 9.4.3. Exploratory Endpoints Analyses** **Section 10.10. Appendix 10**			
Section 8.1.1 Factor FVIII activity **Section 8.1.3. Bleeding episodes and FVIII concentrates**	As implemented via the PACL dated 09Jun2022: Clarifications on which data (bleeds and infusions) have to be collected in eDiary in the event a participant resumes prophylaxis after Month 24 visit. Clarifications with regards to the frequency of FVIII assessments in the setting of elevated FVIII activity levels >150%.	The revisions on bleeds and FVIII infusions collection are to limit participants' burden when they resume prophylaxis. The revisions on FVIII activity assessments are for clarity.	Nonsubstantial
Section 1.3 Schedule of Activities	Clarification that assessment of anti-AAV antibodies to AAV6 (total and neutralizing) is performed yearly which includes week 52.	This revision is to add a missing cross in the schedule of activities.	Nonsubstantial
Section 10.1.1 Regulatory and Ethical Considerations **Section 10.1.3 Informed Consent Process** **Section 10.1.4 Data Protection**	Clarifications	Updates made for consistency with the sponsor's protocol template.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial or Nonsubstantial
Section 10.1.5 Dissemination of Clinical Study Data **Section 10.1.6 Data Quality Assurance** **Section 10.1.8 Study and Site Start and Closure** **Section 10.1.9 Publication Policy**			

This amendment incorporates all revisions to date, including amendments made at the requests of country health authorities and IRBs/ECs.

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1.1. Synopsis

Protocol Title: A PHASE 1/2, OPEN-LABEL, ADAPTIVE, DOSE-RANGING STUDY TO ASSESS THE SAFETY AND TOLERABILITY OF SB-525 (PF-07055480) (RECOMBINANT AAV2/6 HUMAN FACTOR 8 GENE THERAPY) IN ADULT SUBJECTS WITH SEVERE HEMOPHILIA A

Short Title: Phase 1/2 Dose-Ranging Study of Recombinant AAV2/6 Human Factor VIII Gene Therapy SB-525 (Pfizer Reference: PF-07055480) in Adult Participants with Severe Hemophilia A.

Rationale:
Hemophilia A is an X linked blood coagulation disorder caused by mutations of the Factor 8 gene (F8), which encodes the Factor VIII (FVIII) clotting protein. Activated FVIII associates with activated Factor IXa to form the intrinsic Factor Xase complex, a complex that is critical for the intrinsic clotting pathway. Patients with severe hemophilia A have FVIII activity of less than 1% of normal (<1 IU/dL; equivalent to 0.01U/mL).

Restoration of production of functional FVIII to >1% of normal levels can reduce or eliminate the need for prophylactic treatment with FVIII concentrate, and, if at or above 5% of normal activity, can substantially reduce hemorrhage following all but the most severe trauma. However, chronic, repeated intravenous (IV) treatment is required for prophylaxis against spontaneous hemorrhage. Acute hemarthrosis usually requires access to several repeated IV infusions of FVIII until the bleeding stops.

The proposed SB-525 (PF-07055480) clinical study uses a recombinant adeno-associated vector 2/6 (AAV2/6) encoding the complementary deoxyribonucleic acid (cDNA) for the B-domain deleted (BDD) human F8 gene (hF8). The SB-525 (PF-07055480) vector encodes a liver-specific promotor module and AAV2/6 exhibits liver tropism, thus providing the potential for long-term hepatic production of FVIII in participants with hemophilia A.

Objectives and Endpoints

Objectives	Endpoints
Primary objective	Primary endpoints
• To evaluate the safety and tolerability of SB-525 (PF-07055480)	• Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) including clinically significant changes in physical examination, clinical laboratory assessments, immune parameters, vital signs, ECG, liver imaging

Objectives	Endpoints
• To evaluate the time-course profile of FVIII activity after dosing with SB-525 (PF-07055480).	• Changes in circulating FVIII activity
Secondary objectives	Secondary endpoints
• To evaluate clinical impact (efficacy and quality of life) on hemophilia after dosing with SB-525 (PF-07055480).	• Change from baseline in use of FVIII replacement therapy and frequency and severity of bleeding episodes • Change from baseline in the EQ-5D-5L
• To evaluate immune response to FVIII.	• Measurements of FVIII inhibitor levels
• To evaluate vector shedding of AAV2/6.	• Detection of AAV2/6 vector DNA by PCR in plasma, saliva, urine, stool and semen
Exploratory objectives	Exploratory endpoints
• To evaluate the concurrence between FVIII levels by ELISA (FVIII antigen) and by FVIII activity assays	• Measurements of FVIII antigen levels
• To further investigate SB-525 (PF-07055480) mechanism of action and the immune response	• Measurements of neutralizing activity and antibodies to AAV2/6, as well as T-cell responses to AAV2/6 and FVIII • Measurements of von Willebrand factor (vWF), C-reactive protein (CRP), and IL6
Optional liver biopsy substudy only: • To evaluate vector integration in the liver • To evaluate the histopathology of the liver tissue • To assess the expression of protein and/or RNA levels of FVIII and other biomarkers of interest in the liver	• For the integrations analyses (as feasible, i.e. depending on the quantity of biological material collected): the number and location of integration sites, the location of the integration sites relative to transcription start sites, the nature of the inserted sequence, the frequency of insertions, and the frequency and distribution for each size and type of insertion • Other exploratory endpoints (as feasible): histopathology assessment (eg. presence of fibrosis assessment, presence of lymphocytic invasion), protein and/or

Objectives	Endpoints
	RNA expression of FVIII and selected biomarkers (eg. Grp78, Gal3BP)

Overall Design:
SB-525-1603 (C3731001) is a phase 1/2, open-label, single-dose, dose-ranging study. The dose selection and number of participants studied at each dose level will be based on safety and kinetics of circulating FVIII levels observed in previously dosed participants.

Number of Participants:
Approximately 20 participants may be enrolled in this study. The dose selection and number of participants studied at each dose level will be based on safety and the cumulative kinetics of circulating FVIII levels observed in previously dosed participants.

Intervention Groups and Duration:
The possible clinical dose level range will be from 6×10^{11} vg/kg to 6×10^{13} vg/kg, with a starting dose of 9×10^{11} vg/kg, which is expected to yield >5% normal FVIII levels in 57% of treated participants. Several dose levels may need to be studied to identify a safe and tolerable therapeutic range. If observed FVIII levels at the starting dose level of 9×10^{11} vg/kg are higher than anticipated, then dose de-escalation to 6×10^{11} vg/kg will be considered. The planned dose escalation interval between dose levels is roughly 2-fold (0.33 log).

Dose levels considered include:

Possible Total rAAV Dose (vg/kg)
6×10^{11}
9×10^{11}
1.2×10^{12}
2×10^{12}
4×10^{12}
6×10^{12}
1×10^{13}
2×10^{13}
3×10^{13}
4×10^{13}
5×10^{13}
6×10^{13}

The duration of study participation will be approximately 62 months for each participant, divided into approximately 8 weeks (2 months) for screening, and 60 months for treatment and study follow-up. Accrual is planned for 20 months.

Data Monitoring Committee: Yes

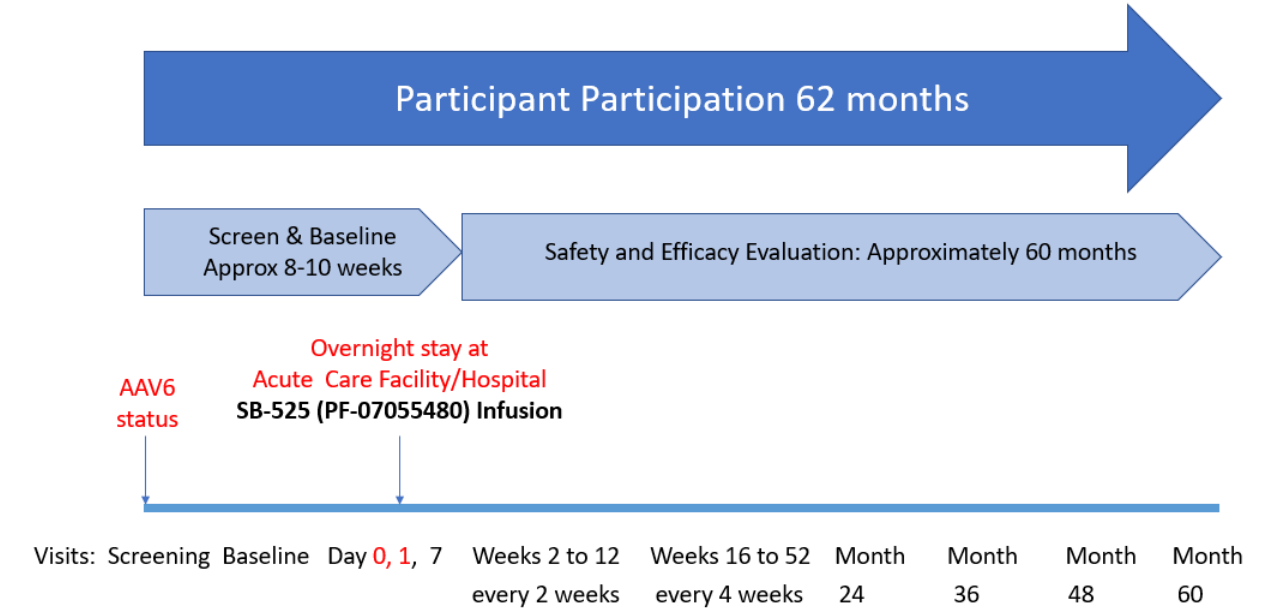
Statistical Methods

Analyses will be descriptive and exploratory in nature.

All analyses of safety will be performed on the safety population, and participants will be analyzed according to the actual treatment they received. Efficacy analyses will be performed using descriptive methods. No formal statistical analysis of study data is planned.

1.2. Schema

Figure 1 C3731001 Study Design



Note: From Week 52 to Month 60, protocol defined laboratory assessments (Table 1) will be collected every 6 months.

1.3. Schedule of Activities (SoA)

Table 1 provides an overview of the protocol visits and procedures. Refer to the Study Assessments and Procedures section of the protocol for detailed information on each procedure and assessment required for compliance with the protocol. The investigator may schedule visits (unplanned visits) in addition to those listed in Table 1, in order to conduct evaluations or assessments required to protect the well-being of the participant.

Table 1. Schedule of Activities

Procedure	Screening	Baseline[a]	Infusion Center		Day 7 (±1 day)	Weeks 2, 4, 6, 8, 10, 12 (±3 days)	Weeks 16, 20, 24, 28, 32, 36, 40, 44, 48, 52 (±1 wk)	Months 24, 36, 48, 60 (±1 M)	Early Termination Visit
			Day 0[b]	Day 1					
Informed consent	X								
Neutralizing activity to AAV2/6	X[c]								
Medical history, inclusion/exclusion criteria	X								
Chest X-ray[d]	X								
Urinalysis with microscopic examination	X								
Liver elastography[d,e]	X								
Hepatitis B panel, HCV antibody, HCV RNA viral load, and HIV RNA viral load and HIV-1/2 antibody assay	X								
FVIII genome sequencing[f]	X								
Circulating alpha fetoprotein level[g]	X					X (wk 12 only)	X (wks 24 and 52)	X	X
Liver MRI[d,g]	X					X (wk 12 only)	X (wks 24 and 52)	X	X
12-lead ECG[d]	X						X (wk 52)	X	
Physical examination including vital signs, height, and weight[h]	X	X (weight only)			X	X (wks 4, 8, 12)	X (wks 20, 28, 36, 44, 52)	X	X

Table 1. Schedule of Activities

Procedure	Screening	Baseline[a]	Infusion Center		Day 7 (±1 day)	Weeks 2, 4, 6, 8, 10, 12 (±3 days)	Weeks 16, 20, 24, 28, 32, 36, 40, 44, 48, 52 (±1 wk)	Months 24, 36, 48, 60 (±1 M)	Early Termination Visit
			Day 0[b]	Day 1					
Vital Signs[q] when not already included with physical examination			X	X					
Concomitant medication review	X	X	X	X	X	X	X	X[t]	X
CBC with differential and platelet count	X	X	X	X		X (wks 4, 8, 12)	X (wks 28, 52)	X	X
Serum chemistry	X	X	X	X		X	X	X	X
Liver panel[i]	X	X			X (2x in first wk)	X (2x/wk)	X[j] (2x/wk, wks 13-20)	X (every 6m from Wk52 to Mth60)	X
Coagulation screen (PT, INR, and aPTT)	X	X	X			X (wks 4, 8, 12)	X	X	X
Anti-AAV antibodies to AAV2/6 (total and neutralizing)		X				X (wk 4 only)	X (wk 52 only)	X	
FVIII activity[k,m]	X	X		X	X	X (1x/wk)	X (1x/wk through Wk 20) [l]	X (every 6m from Wk52 to Mth60)	X
FVIII antigen[v]	X	X		X	X	X (1x/wk)	X[l]	X	X
FVIII inhibitor titer[n]	X	X		X		X[l] (wks 4, 8, 12)	X[l]	X (every 6m from Wk52 to Mth60)	X
EQ-5D-5L		X				X (wk 12 only)	X (wks 24, 52)	X	X
sEGFR and Gal3BP		X							
vWF and plasma banking		X			X	X (wks 4, 8, 12)	X	X	X

Table 1. Schedule of Activities

Procedure	Screening	Baseline[a]	Infusion Center		Day 7 (±1 day)	Weeks 2, 4, 6, 8, 10, 12 (±3 days)	Weeks 16, 20, 24, 28, 32, 36, 40, 44, 48, 52 (±1 wk)	Months 24, 36, 48, 60 (±1 M)	Early Termination Visit
			Day 0[b]	Day 1					
CRP/IL6		X			X				
AAV2/6 vector genome in plasma, saliva, urine, stool, and semen by PCR[o]		X	X (plasma only)		X	X (wks 2, 4, 8, 12)	X[p]	X[p]	X
Assessment of bleeding episodes and FVIII concentrates usage	This is an ongoing collection and review.								
Abdominal examination for hepatomegaly		X				X	X	X	X
AE(s) assessment	X	X	X	X	X	X	X	X	X
SB-525 (PF-07055480) infusion			X						
Interferon-gamma ELISPOT[r]		X				X (wk 6)	X[u]	X[u]	
Interim phone contact[s]								X (Months 30, 42 and 54)	
Optional Liver Biopsy[w]					X (postinfusion)				

a. Baseline assessments to be performed approximately 1 week prior to SB-525 (PF-07055480) infusion.
b. Laboratory samples to be collected prior to dosing.
c. Following informed consent, the screening process starts with assessing the neutralizing activity to AAV2/6. It is recommended to perform the other screening tests only if the AAV2/6 neutralizing activity results are acceptable. The neutralizing activity is to be reassessed if the screening period takes more than 8 weeks.
d. If a participant had this procedure conducted within 6 months prior to screening, then it can be used in place of the screening assessment.
e. Liver elastography (or any equivalent imaging technology) required for any participant with a history of hepatitis C or suspicion of chronic liver disease to evaluate for fibrosis. Also see footnote c.
f. Only for participants with no FVIII gene sequencing previously.
g. For every participant. However, any participant treated with SB-525 (PF-07055480) who has an elevated alpha fetoprotein and MRI mass greater than 2 cm or suspicious for HCC will undergo biopsy using surgical doses of Factor VIII replacement, if needed. An MRI of the liver can be replaced by an abdomen CT-scan (or any equivalent imaging technology) – with vascular phases specific for a search of a liver mass – in case the participant has a contraindication to MRI (for example history of hip replacement).
h. Height (cm) will be collected at screening only, and weight (kg) will be collected at screening and baseline only.

Table 1. Schedule of Activities

Procedure	Screening	Baseline[a]	Infusion Center		Day 7 (±1 day)	Weeks 2, 4, 6, 8, 10, 12 (±3 days)	Weeks 16, 20, 24, 28, 32, 36, 40, 44, 48, 52 (±1 wk)	Months 24, 36, 48, 60 (±1 M)	Early Termination Visit
			Day 0[b]	Day 1					

i. Both local and central LFTs will be performed. At Screening, liver panel will be performed twice, at least 1 week apart. Following infusion, LFTs will be done twice a week from the first collection approximately 3 to 4 days after Day 0 and continuing until Week 20. The local laboratory results may be utilized for the investigator to react promptly if an ALT elevation occurs. Note: Local and central LFTs are drawn tandemly twice weekly.
j. Continue central and local LFT assessments twice per week if participant is receiving steroids. LFT assessments will continue twice weekly until 3 consecutive results demonstrate stable liver function. At this time LFT assessment may be decreased to once a week.
k. If a participant has FVIII administration around the day of a visit when FVIII activity is scheduled, sample collection should be postponed at least 48 or 72 hours based on the FVIII product used (short half-life or long half-life, respectively).
l. If a participant is receiving steroids or if a participant develops an inhibitor, weekly assessments of FVIII activity, FVIII antigen and FVIII inhibitor titer will continue.
m. Central and local FVIII activity assessment will be performed. After Week 52, measurement of FVIII activity will be done every 6 months, and may increase if warranted by changes in FVIII activity or presence of inhibitor. Mobile phlebotomy can be used as needed.
n. Central FVIII inhibitor titer assessment will be conducted. After Week 52, measurement of FVIII inhibitor titer may increase if warranted by changes in FVIII activity or the presence of inhibitor.
o. Vector genomes in plasma, saliva, urine, stool, and semen by PCR (unless three previous consecutive specimens have been negative, see footnote p). On Day 0, vector genome titer should be obtained in **plasma only** at 12 hours from the start of the infusion ± 1 hour.
p. Collection will only continue until three consecutive negative samples are obtained on a sample-type basis and every 1 month after Week 52 to Month 60 [±1 month]). For example, after urine sample results are negative 3 consecutive times, the urine sample will stop being collected while the other samples may continue if they are positive.
q. Vital signs to be collected at various times from pre-dose until discharge as described in Section 8.2.2.
r. Additional samples will be collected if there is a 50% drop in Factor VIII expression, and/or if possible prior to initiation of a corticosteroid treatment.
s. Follow-up phone calls to be conducted every 6 months through Month 60 starting 6 months after Month 24 visit between yearly visits (Months 30, 42 and 54) to review AEs, bleeding events and concomitant medications. Telephone contacts are not required if they fall when a participant is due on site for a blood draw (Months 36 and 60).
t. As per concomitant medication reporting requirements as described in Section 6.5.
u. As clinically warranted.
v. FVIII antigen testing will be conducted only at central lab for all time points and as clinically necessary.
w. Participants who consent to participate in this optional substudy will undergo liver biopsy. The biopsy may be performed at any time postinfusion when deemed appropriate by the investigator, and according to Section 8.2.14.

2. INTRODUCTION

SB-525 (PF-07055480) is an AAV2/6 vector encoding the hF8 cDNA that is currently being investigated in participants with severe Hemophilia A. SB-525 is legacy nomenclature referring to SB-525 (PF-07055480) and may be utilized interchangeably within this protocol.

2.1. Study Rationale

The purpose of this study is to evaluate the safety and tolerability of SB-525 (PF-07055480) and the FVIII activity by increasing dose levels.

2.2. Background

2.2.1. Hemophilia A

Hemophilia A, the most common form of hemophilia, occurs in 1 in 5000 males and makes up about 80% of the 20,000 individuals in the United States with hemophilia.[1] Hemophilia A is an X linked blood coagulation disorder caused by mutations of the F8 gene, which encodes the FVIII clotting protein. Activated FVIII associates with activated Factor IXa to form the intrinsic Factor Xase complex, a complex that is critical for the intrinsic clotting pathway. Patients with severe hemophilia A have FVIII activity of less than 1% of normal (<1 IU/dL; equivalent to <0.01 IU/mL). If untreated, they suffer frequent hemorrhage into joints or muscles, either spontaneously or in response to mild trauma resulting in crippling arthropathy or premature death.

Restoration of production of functional FVIII to >1% of normal levels can reduce or eliminate the need for prophylactic treatment with FVIII concentrate, and, if at or above 5% of normal activity, can substantially reduce hemorrhage following all but the most severe trauma. Chronic, repeated IV treatment is currently required for prophylaxis against spontaneous hemorrhage. Acute hemarthrosis usually requires access to several repeated IV infusions of FVIII until the bleeding stops. FVIII administered as a protein replacement therapy has a short half-life (12 hours) requiring frequent IV injections (approximately 3 times/week) to maintain hemostatically effective levels. FVIII replacement products with longer half-lives have recently been commercialized, but they still require frequent infusions due to only modest improvement in half-life (approximately 1.5-fold over standard replacement therapy). Furthermore, the development of inhibitors to administered FVIII is a complication in 25-30% of severe hemophiliacs; in these inhibitor patients, hemostatic bypass agents can be used to treat or prevent bleeding and repeated infusions of FVIII, often over many months, can be used to induce immune tolerance and reduce inhibitor levels.

The rationale for the proposed clinical therapy is to reduce or eliminate the need for FVIII replacement therapy. This study uses a recombinant AAV2/6 encoding the cDNA for the BDD hF8. The secreted FVIII has the same amino acid sequence as approved recombinant anti-hemophilic factors (ReFacto AF and Xyntha). The SB-525 (PF-07055480) vector encodes a liver-specific promotor module and AAV2/6 exhibits liver tropism, thus providing the potential for long-term hepatic production of FVIII in patients with hemophilia A.

Preclinical studies in hemophilia A mice, normal mice and non-human primates (NHP) treated with SB-525 (PF-07055480) demonstrated clinically relevant levels of circulating

human FVIII. Bleeding time was significantly reduced following IV administration of SB-525 (PF-07055480) to hemophilia A mice. Normal mice and NHPs administered SB-525 (PF-07055480) IV and evaluated over 3 months and 2 months, respectively, showed no adverse effects related to treatment. There were no tumors detected or evidence of carcinogenicity in either species. SB-525 (PF-07055480) was shown to preferentially target the liver compared to other tissue types. The highest dose levels tested in mouse and NHP studies were 22-fold and 6.7-fold higher, respectively, than the anticipated clinical starting dose of 9×10^{11} vg/kg.

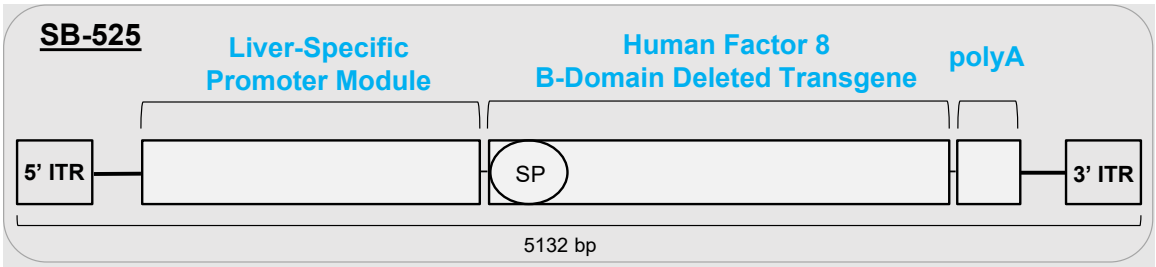
The constant production of FVIII after a single SB-525 (PF-07055480) administration may provide potential benefit in durable protection against bleeding and the complications thereof without lifelong repetitive IV factor replacement administration.

2.2.2. SB-525 (PF-07055480) Molecular Design and Construction

Gene therapy approaches in hemophilia A have been historically constrained by the large size of the hF8 gene, the high AAV dose required to achieve therapeutic FVIII levels, and the low manufacturing yields of AAV hF8. The study intervention SB-525 (PF-07055480), however, has a shorter coding sequence for hF8 (the hF8 BDD), an optimized, robust liver-specific promoter module to drive hF8 expression, and improved virus yields.

SB-525 (PF-07055480) is an AAV2/6 vector encoding the hF8 cDNA (Figure 2). The SB-525 (PF-07055480) hF8 cDNA expression cassette consists of liver-specific regulatory elements that drive expression of the hF8 transgene. The hF8 transgene is under the control of a liver-specific promoter module that is specifically and highly active in the liver, the intended target tissue, but inactive in non-liver cells and tissue types, which prevents the expression and activity of FVIII in non-target tissues. The hF8 transgene comprises a codon-optimized hF8 BDD construct, including the native hF8 signal peptide (SP) and is flanked by AAV serotype 2 inverted terminal repeats (ITRs). Importantly, the secreted FVIII has the same amino acid sequence as approved recombinant anti-hemophilic factors (ReFacto AF and Xyntha).

Figure 2 SB-525 (PF-07055480) cDNA Construct



2.2.3. Preclinical Studies

The preclinical studies demonstrated that treatment with PF-07055480 resulted in circulating levels of biologically active human FVIII that appear sufficient to have an efficacious impact in human subjects. Toxicity studies did not show any adverse findings of concerns for use in human patients.

Detailed information about preclinical studies with SB-525 (PF-07055480) is provided in the Investigator's Brochure.

2.3. Benefit Risk Assessment

Detailed information about the known and expected benefits and risks and reasonably expected AEs of SB-525 (PF-07055480) may be found in the investigator's brochure, which is the single reference safety document (SRSD) for this study.

3. OBJECTIVES, ESTIMANDS AND ENDPOINTS

Table 2. Objectives and Endpoints

Objectives	Endpoints
Primary objective	Primary endpoints
• To evaluate the safety and tolerability of SB-525 (PF-07055480).	• Incidence of AEs and SAEs including clinically significant changes in physical examination, clinical laboratory assessments, immune parameters, vital signs, ECG, liver imaging
• To evaluate the time-course profile of FVIII activity after dosing with SB-525 (PF-07055480).	• Changes in circulating FVIII activity
Secondary objectives	Secondary endpoints
• To evaluate clinical impact (efficacy and quality of life) on hemophilia after dosing with SB-525 (PF-07055480).	• Change from baseline in use of FVIII replacement therapy and frequency and severity of bleeding episodes • Change from baseline in the EQ-5D-5L
• To evaluate immune response to FVIII.	• Measurements of FVIII inhibitor levels
• To evaluate vector shedding of AAV2/6.	• Detection of AAV2/6 vector DNA by PCR in plasma, saliva, urine, stool and semen
Exploratory objectives	Exploratory endpoints
• To evaluate the concurrence between FVIII levels by ELISA (FVIII antigen) and by FVIII activity assays.	• Measurements of FVIII antigen levels

Table 2. Objectives and Endpoints

Objectives	Endpoints
• To further investigate SB-525 (PF-07055480) mechanism of action and the immune response.	• Measurements of neutralizing activity and antibodies to AAV2/6, T-cell responses to AAV2/6 and FVIII • Measurements of von Willebrand factor (vWF), C-reactive protein (CRP), and IL6
Optional liver biopsy substudy only: • To evaluate vector integration in the liver • To evaluate the histopathology of the liver tissue • To assess the expression of protein and/or RNA levels of FVIII and other biomarkers of interest in the liver	• For the integrations analyses (as feasible, i.e, depending on the quantity of biological material collected): the number and location of integration sites, the location of the integration sites relative to transcription start sites, the nature of the inserted sequence, the frequency of insertions, and the frequency and distribution for each size and type of insertion • Other exploratory endpoints (as feasible): histopathology assessment (eg, presence of fibrosis assessment, presence of lymphocytic invasion), protein and/or RNA expression of FVIII and selected biomarkers (eg, Grp78, Gal3BP)

4. STUDY DESIGN

4.1. Overall Design

This study is a phase 1/2, open-label, single-dose, dose-ranging study. The dose selection and number of participants studied at each dose level will be based on safety and kinetics of circulating FVIII levels observed in previously dosed participants.

The adaptive design of this phase 1/2 study assigns participants to SB-525 (PF-07055480) dose levels based on the average of FVIII expression in the 2 first participants of a cohort.

For each cohort, the first dosed participant will be followed for 6 weeks before a second participant can be dosed. After the second participant in a cohort is followed for 6 weeks, the Data Monitoring Committee (DMC) will meet and recommend either a dose escalation, dose de-escalation, or cohort expansion (with potential reassessment of participant's data) based on their review of the cumulative data available from the first 2 participants of a cohort.

The duration of study participation will be approximately 62 months for each participant, divided into approximately 8 weeks (2 months) for screening, and 60 months for treatment and study follow-up.

4.1.1. Screening Period

In order to facilitate enrollment and decrease unnecessary blood sampling, it is recommended to start the screening process with the assessment of the AAV2/6 serological status only (Section 8.2.13.1). If the participant qualifies, then the rest of the screening tests (total duration approximately 8 weeks) will be performed.

All screening assessments are listed in the SoA table (Table 1). A participant must meet all Inclusion Criteria, and none of the Exclusion Criteria, to participate in this study.

4.1.2. Treatment Period

This study will follow an adaptive, dose escalation and participant enrollment design to establish and enroll participants at a dose level that can achieve therapeutically useful FVIII levels comparable with emerging gene therapies (in the 10-150% range), with a therapeutic goal of FVIII activity of 40-100% of normal. The schema aims to minimize enrollment to dose levels that are inactive or results in FVIII levels of >150%.

One (1) participant will initially be enrolled at a given dose level and followed over 6 weeks. After review of safety data for the first participant, a second participant will be enrolled at the same dose level and also followed for 6 weeks. Then DMC will meet and recommend dose escalation, dose de-escalation, or cohort expansion.

The possible clinical dose level range will be from 6×10^{11} vg/kg to 6×10^{13} vg/kg, with a starting dose of 9×10^{11} vg/kg, which is expected to yield >5% normal FVIII levels in 57% of treated participants. Several dose levels may need to be studied to identify a safe and tolerable therapeutic range. If observed FVIII levels at the starting dose level of 9×10^{11} vg/kg are higher than anticipated, then dose de-escalation to 6×10^{11} vg/kg will be considered. The planned dose escalation interval between dose levels is roughly 2-fold (0.33 log).

Participants will be dosed with one of 12 potential dose levels, as outlined in the table below:

Possible Total rAAV Dose (vg/kg)
6×10^{11}
9×10^{11}
1.2×10^{12}
2×10^{12}
4×10^{12}
6×10^{12}
1×10^{13}
2×10^{13}
3×10^{13}
4×10^{13}

Possible Total rAAV Dose (vg/kg)
5×10^{13}
6×10^{13}

The starting dose level (9×10^{11} vg/kg) was associated with FVIII activity of 12% of normal in a NHP study. Unless more are recommended by the DMC, approximately 20 participants are planned to be dosed in this clinical trial.

Based on DMC review of safety and pharmacodynamics (PD) data, additional participants may be enrolled at a specific dose level. The dose selected will be a tolerable dose level where FVIII activity does not exceed 150% based on observed activity and variability.

4.1.2.1. Dose Escalation Based on Pharmacodynamic Response

The dose escalation plan and participant assignment to dose levels will be according to an analysis of FVIII expression response based on weekly longitudinal FVIII activity measurements. At the end of the first cohort, safety and expression data will be available for 2 participants over 12 and 6 weeks, respectively. The DMC will review all the safety data and will recommend, based on this review and the expression levels, to move to a different dose level, de-escalate or expand the current one.

While these guidelines use mean FVIII activity results within dose levels, the DMC must also be consulted if any participant at a given dose level has FVIII peak activity of >150% prior to adding further participants.

4.1.2.2. Dose Escalation Based on Safety

Determination of Dose limiting toxicities (DLTs) will be made by the DMC (Section 9.6). Further enrollment to a dose level and dose escalation based on PD response per the above schema will be modified for reasons of safety based on the guidelines below:

- If at any given dose level, >50% participants experience a DLT, additional participants can only be enrolled at that dose level in concert with the DMC.
- Escalation to a higher dose level can only occur if not more than 40% of participants, and at least 2 participants experience a DLT.
- An average FVIII expression of 150% or more will be considered as a safety issue and the DMC may consider dose de-escalation.

Safety data will take precedence over expression data for dose escalation. If further enrollment in a cohort for safety reasons is prevented by insufficient expression data, the DMC and sponsor will adjudicate the circumstances for further participant enrollment.

4.1.3. Early Termination

Participants who discontinue from the study prematurely or are withdrawn from the study will be asked to return to the study site for an Early Termination Visit (ETV). It is at the

discretion of the investigator to waive any procedures if they have been performed as part of standard of care within the standard interval of scheduled study visits per protocol. See the list of assessments to be performed at the ETV in the SoA (Table 1).

The investigator must make and document every attempt to ensure all participants are followed through the end of study (Month 60).

4.2. Scientific Rationale for Study Design

Hemophilia is an attractive target for gene therapy. This is due in part because minute-to-minute regulation of FVIII/Factor IX clotting protein (FIX) expression is not required (unlike proteins such as insulin). In addition, there is a wide therapeutic window for FVIII/FIX expression since therapeutic benefit can be achieved in severe hemophiliacs, who have FVIII/FIX activity <1%, with only modest improvements in factor expression. Any factor activity over 1% results in an improved phenotype (moderate hemophilia 1-5% or mild hemophilia 5-40%).

As the study was being planned, 1 adeno-associated virus (AAV)-mediated FVIII cDNA- and 5 AAV-mediated FIX cDNA-gene-transfer studies have been initiated and have reported at least some clinical study data. Details are provided in the Investigator's Brochure. Of the various AAV serotypes tested in the clinic, none have utilized the AAV6 serotype that will be tested in this clinical trial. Of the 4 studies where gene therapy was delivered intravenously, factor protein expression generally exhibited a dose response, though peak expression and durability of effect have been variable. Alanine aminotransferase (ALT) elevations due to AAV-associated transaminitis have been observed in at least 1 dose level in all studies reported to date using the IV route. AAV-associated transaminitis has been reported at vector doses ranging from 5×10^{11} vg/kg to 6×10^{13} vg/kg, with variable amount of losses in factor protein expression that may be associated with time to initiation of steroid therapy. Additional information on AAV-associated transaminitis is provided in Section 10.7.

4.2.1. Rationale for Use of rAAV2/6 Serotype Based on Preclinical Studies

The rAAV2/6 serotype was selected for use in Sangamo gene therapy platforms based on previous NHP data showing that AAV2/6 was primarily hepatotrophic, with similar biodistribution to AAV2/8 and that AAV2/6 and AAV2/8 vectors yielded similar levels of circulating FIX transgene expression (see the Investigator's Brochure).

4.3. Justification for Dose

The rationale for SB-525 (PF-07055480) dose selection was based on (i) the dose-response relationship of circulating levels of human FVIII generated in NHP following a single IV dose administration of SB-525 (PF-07055480), (Sangamo Study Number TX16-HMA-008); (ii) preclinical dose ranging safety studies (2×10^{11} to 2×10^{13} vg/kg) in mice and NHP resulting in no adverse events (AEs), histological changes or tumorigenicity; and (iii) recently published clinical trials involving similar cDNA-based strategies (see the Investigator's Brochure). The proposed clinical dose range is 6×10^{11} to 6×10^{13} vg/kg, with a starting dose of 9×10^{11} vg/ kg, which was associated with FVIII activity of 12% of normal

in an NHP study. The 6×10^{11} dose is an optional de-escalation dose level if FVIII activity in humans is much higher than observed in NHPs.

Although the translation of SB-525 (PF-07055480) pharmacodynamics (PD); delivery to liver, transduction, and FVIII expression) from NHP to humans is unknown, the dose escalation assumes a direct translation between species with starting low dose levels expected to result in therapeutic FVIII levels. Subsequent doses will follow an adaptive dose escalation or de-escalation plan to mitigate the possibility that the human efficacious dose is higher than observed in NHP.

A population kinetic-PD model incorporated the monkey plasma human FVIII data with the assumption that the NHP response models human response in terms of plasma FVIII levels. With this model, a clinical starting dose of 9×10^{11} vg/kg is expected to yield >5% normal FVIII levels in 57% of treated participants. This is a reasonable target for the starting dose. Further information of the dose escalation plan is found in Section 4.1.2.

4.4. End of Study Definition

A participant is considered to have completed the study if he has completed all phases of the study including the End-of-Study (EOS) visit (Month 60).

The end of the study is defined as the date of the EOS Visit of the last participant in the study.

5. STUDY POPULATION

This study can fulfill its objectives only if appropriate participants are enrolled. The following eligibility criteria are designed to select participants for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular participant is suitable for this protocol.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Signed informed consent.
2. Male ≥18 years of age able to understand the informed consent.
3. Severe hemophilia A (with past evidence of circulating FVIII activity of <1% normal).
4. Treated or exposed to FVIII concentrates or cryoprecipitate for at least 150 exposure days.
5. ≥12 bleeding episodes if receiving on-demand therapy over the preceding 12 months.

6. Sexually active participants must agree to use double barrier contraceptive (one of them being a condom) or abstinence, and all participants must refrain from donating their sperm until at least 3 consecutive semen samples after SB-525 (PF-07055480) treatment are negative for AAV2/6 and for a minimum of 90 days after SB-525 (PF-07055480) administration.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Neutralizing activity to AAV6 capsid.
2. Current inhibitor, or history of FVIII inhibitor (except for transient low titer inhibitor detected in childhood).
3. History of hypersensitivity response to FVIII.
4. History of hepatitis B or HIV-1/2 infection.
5. History of hepatitis C, unless viral assays in 2 samples, collected at least 6 months apart, are negative.
6. Two (2) or more occurrences of any of the following: hemoglobin <10g/dL, platelets <100,000/µL, or white blood cells less than 4,000 cells/µL.
7. Evidence of any bleeding disorder in addition to hemophilia A.
8. History of tuberculosis or systemic fungal disease.
9. Symptomatic cardiovascular disease as a co-morbid condition.
10. Markers of hepatic inflammation or overt or occult cirrhosis as evidenced by 1 or more of the following:
    - Albumin ≤3.5 g/dL;
    - Total bilirubin >1.5 x ULN and direct bilirubin ≥0.5 mg/dL;
    - Alkaline phosphatase >2.0 × ULN;
    - ALT or aspartate aminotransferase (AST) >1.3 × ULN;
    - International Normalized Ratio (INR) >1.5.
11. History of chronic renal disease or creatinine ≥1.5 mg/dL.
12. Use of systemic (IV or oral) immunomodulatory agent or steroid use within 3 months prior to screening (inhaled or topical treatment is allowed, eg, for asthma or eczema).

13. History of chronic infection or other chronic disorder considered an unacceptable risk.

14. Presence of liver mass on magnetic resonance imaging (MRI) or any equivalent imaging technology, or, positive alpha fetoprotein.

15. Presence of >Grade 2 liver fibrosis on elastography (or any equivalent imaging technology) for participants with history of treated Hepatitis C or suspicion of chronic liver disease.

16. History of malignancy except for successfully treated basal cell carcinoma.

17. History of alcohol or substance abuse.

18. Any contraindication to the use of corticosteroids.

19. Previously received gene therapy product.

20. Participation in prior investigational drug or medical device study within 3 months prior to screening.

21. History of therapeutic non-adherence.

22. Any other reason that, in the opinion of the investigator or medical monitor, would render the participant unsuitable for participation in the study.

5.3. Lifestyle Considerations

Sexually active patients should use 1 of the acceptable forms of contraceptives described in Appendix 4 (Section 10.4).

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants are required to sign a new ICF and will be assigned a different participant number.

6. STUDY INTERVENTION

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

For the purposes of this protocol, study intervention refers to SB-525 (PF-07055480).

6.1. Study Intervention(s) Administered

ARM Name	Single arm study
Intervention Name	PF-07055480
Type	Gene therapy
Dosage Form	Injectable
Dose Strength	6×10^{11}, 9×10^{11}, 1.2×10^{12}, 2×10^{12}, 4×10^{12}, 6×10^{12}, 1×10^{13}, 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13}, 6×10^{13} vg/kg
Dosage	Single infusion Several dose levels may need to be studied to identify a safe and tolerable therapeutic range. For a participant with BMI >30 kg/m^2, dose will be calculated based on an adjusted body weight determination that assumes a maximum permissible BMI of 30 kg/ m^2, eg, for 187.96 cm (6'2") height and 167.8 kg weight (BMI 47.5 kg/m^2) dose will be based on 106.1 kg, which is the weight associated with a BMI of 30 kg/m^2 for a 187.96 cm (6'2") tall individual
Route of Administration	Intravenous infusion/injection
IMP and NIMP	IMP (investigational medicinal product)
Sourcing	Provided centrally by the sponsor.
Packaging and Labeling	Study Intervention will be provided in a 4.7 mL/vial. Each vial will be labeled as required per country requirement.
Current/Former Name(s) or Alias(es)	PF-07055480 SB-525 Adeno-associated viral vector with human factor VIII gene

6.1.1. Administration

Side effects following SB-525 (PF-07055480) administration may include transient fever, chills, and/or nausea. It is recommended that the participant should be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride (Benadryl) 25-50 mg by mouth or IV approximately 1 hour prior to the administration of SB-525 (PF-07055480). These medications may be repeated every 3 - 4 hours as needed. NSAIDs are not recommended because of the potential to cause platelet dysfunction but may be used at the discretion of the investigator.

It is recommended that the participant also fast for 2 hours after the administration. All participants will be switched to an on-demand regimen approximately 2 weeks after infusion and subsequently modulate their FVIII replacement use according to their needs.

SB-525 (PF-07055480) will be injected IV using a syringe pump or IV infusion pump (FDA approved delivery system as described in the Study Investigational Product [IP] Manual). Total volumes will be dependent on participant's cohort assignment and body weight (kg) at baseline. SB-525 (PF-07055480) will be administered through an IV catheter at a controlled

speed while monitoring the participant's vital signs (heart rate, blood pressure [BP], respiratory rate [RR], and temperature). Detailed instructions for the thaw and administration of the investigational product are in the Study IP Manual.

Participants will be admitted to the infusion center facility for SB-525 (PF-07055480) infusion and will remain in the infusion center for 24 hours after the end of infusion for observation and will be discharged when all AEs, concomitant medications, and all vital signs (temperature, heart rate [HR], RR, and BP) are stable.

All participants will be asked to return to the study site for a follow-up visit 7 ± 1 days after SB-525 (PF-07055480) infusion. All safety data must be reviewed prior to discharge.

6.2. Preparation/Handling/Storage/Accountability

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

- Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

- Further guidance and information for the final disposition of unused study interventions are provided in the IP Manual.

Ordering the IP and Preparation: Details are provided in the IP Manual and it should be reviewed carefully. Once eligibility has been confirmed, the IP can be ordered noting that it will take approximately 3 weeks for product delivery.

6.3. Measures to Minimize Bias: Randomization and Blinding

Allocation of study intervention	This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an IRT system (eg, IMPALA). The site will contact the IRT system approximately 3 weeks before the start of study intervention administration for each participant. The infusion site will record the intervention assignment on the applicable case report form, if required.

6.4. Study Intervention Compliance

Study treatment will be infused via infusion pump on Day 0 under supervision by the site staff. The vial lot number, total volume, and infusion time (start and stop times) will be monitored and recorded by the site staff. Full compliance with study treatment infusion is anticipated.

Administration: If a participant does not receive a complete infusion for any reason, she or he will not be rescreened, but will be followed for safety.

6.5. Concomitant Therapy

All concomitant medications and therapy taken through Month 36 must be captured in the CRF. After Month 36, only concomitant medications and therapy associated with SAEs, AEs related to the study drug, or to AEs with an unknown cause will be reported through Month 60 or the ETV, if applicable. See Section 8.3.1 for AE reporting requirements.

The investigator will record all concomitant medications including those given in treatment of AEs on the concomitant medication page in the participant's CRF. Any medication taken by the participant from 30 days prior to screening throughout the course of the study, including over-the-counter medicinal products, dietary supplements, and herbal medications, should be recorded on this form. A short course of prednisone may be instituted either as prophylaxis or post AAV exposure if an immune reaction to the AAV capsid develops (Section 10.7). Any treatment with prednisone and pre-treatment with acetaminophen and diphenhydramine hydrochloride should be recorded on the concomitant medications page.

Participants enrolled in this study who previously received prophylactic FVIII replacement regimens will be required to switch to on-demand therapy approximately 2 weeks after infusion with SB-525 (PF-07055480), pending data shows it is safe to do so. The use and timing of FVIII replacement will be monitored closely in this study and will influence the timing of PD assessments for FVIII activity. There is no washout period per se, but all participants will be switched to an on-demand regimen approximately 2 weeks after infusion and subsequently modulate their FVIII replacement use according to their needs. All participants are to carefully record the date and time of each FVIII administration in an e-Diary, so that the elapsed time between their last FVIII administration and each FVIII level collection can be collected. For participants using short half-life replacement products, the resulting FVIII levels are valid only if elapsed time is more than 48 hours. For participants using extended half-life FVIII replacement products, this time is extended to 72 hours. Of note, 48 and 72 hours are reference values, these times can be modified depending on the participant profile (slow versus fast metabolizers) and the prescribing information of each product. The investigator may recommend a different time for his/her study participant after discussion with the medical monitor.

6.5.1. Allowed Therapy

All medications (such as FVIII) with the exception of those that are potentially hepatotoxic are permitted.

6.5.2. Disallowed Therapy

Hepatotoxic agents such as diclofenac, amiodarone, chlorpromazine, fluconazole, isoniazid, rifampin, valproic acid, and high doses of acetaminophen (maximum 4 gm/day) or celecoxib (>100 mg/day) are to be avoided. Participants should be counseled to moderate alcohol consumption throughout the trial (not more than 1 drink per week is suggested); especially for 3 months following dosing with SB-525 (PF-07055480).

6.6. Dose Modification

Within an individual participant, no dose modifications are possible as this is a single administration study.

6.7. Intervention after the End of the Study

No further intervention is planned after the end of the study.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

Since no more than 1 single infusion of SB-525 (PF-07055480) on Day 0 will be administered during the study, this section is not applicable. See Section 4.1 for any disruption in administration of the infusion.

7.1.1. Temporary Discontinuation

Not applicable for this study.

7.1.2. Rechallenge

Not applicable for this study.

7.2. Participant Discontinuation/Withdrawal from the Study

Participants may withdraw or should be discontinued from study for any of the following reasons:

- Request by the participant to withdraw.
- Request of the sponsor or primary care provider if he or she thinks the study is no longer in the best interest of the participant.
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the Institutional Review Board (IRB), Office for Human Research (OHR), Food and Drug Administration (FDA), investigator, or sponsor.

At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted. See the SoA (Table 1) for assessments to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The early discontinuation visit applies only to participants who are enrolled and then are prematurely withdrawn from the study. Participants should be questioned regarding their reason for withdrawal.

If a participant withdraws from the study, he may request destruction of any remaining samples taken and not tested, and the investigator must document any such requests in the site study records and notify the sponsor accordingly.

If the participant withdraws from the study and also withdraws consent (see Section 7.2.1) for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

Lack of completion of all or any of the withdrawal/early termination procedures will not be viewed as protocol deviations so long as the participant's safety was preserved.

7.2.1. Withdrawal of Consent

Withdrawal of Consent occurs when a participant specifically withdraws consent for any further contact with him or persons previously authorized by the participant to provide this information. Participants should notify the investigator in writing of the decision to withdraw consent from future follow-up, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is from study procedures and/or post-treatment study follow-up, and entered on the appropriate CRF page. In the event that vital status (whether the participant is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

7.3. Lost to Follow up

A participant will be considered lost to follow-up if he repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local

equivalent methods). These contact attempts should be documented in the participant's medical record.

- Should the participant continue to be unreachable, he will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

Screening evaluation may take approximately 8 weeks and may be performed across several visits during the screening period. Screening and Baseline may be approximately 10 weeks in duration. Assessments must be completed, and the results reviewed to confirm eligibility. Tests to be performed at screening and baseline are described in the SoA (Table 1).

The investigator (or an appropriate delegate at the investigator site) must obtain a signed and dated ICD before performing any study-specific procedures. ICF and ICD are synonymous and used interchangeably in this protocol.

Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.

Safety issues should be discussed with the sponsor immediately upon occurrence or awareness to determine whether the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Every effort should be made to ensure that the protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator must take all steps necessary to ensure the safety and well-being of the participant. When a protocol-required test cannot be performed, the investigator will document the reason for the missed test and any corrective and preventive actions that he or she has taken to ensure that required processes are adhered to as soon as possible. The study team must be informed of these incidents in a timely manner.

For samples being collected and shipped, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site prior to initiation of the study.

Procedures for performing each assessment not detailed in this protocol should follow local standard of care.

The actual date should be recorded for all procedures and the investigator should make every effort to perform procedures at the scheduled nominal dates.

Planned safety and laboratory assessments may be collected at additional time points following conversation between the investigator and medical monitor based on emerging clinical events. The change in timing for any planned study assessments must be approved and documented by the sponsor, but this will not constitute a protocol amendment. The addition of any new study assessments to the protocol required for any ongoing and new participants would constitute an amendment.

Demographic/Medical History Assessments

The following demographic parameters will be captured: date of birth, sex, race, and ethnicity.

Medical and medication history will be assessed during the screening period as related to the eligibility criteria in Sections 5.1 and 5.2 and will include but is not limited to the following:

- Extensive medical history;
- Medication history (all medications taken within 30 days prior to Screening visit with a particular focus on their FVIII replacement regimen) inclusive of all herbal medications, over-the-counter medicines, protein/high-energy supplements or drinks, weight-loss products, smoking cessation products (inhalers, patches, gum), aspirin (or aspirin-containing products), non-steroidal anti-inflammatory drugs (NSAIDs; or NSAID-containing products), and celecoxib
- History of drug and alcohol use

If the participant is not normally seen at the study site, medical records should be obtained to confirm study eligibility.

8.1. Efficacy Assessments

8.1.1. Factor VIII activity

FVIII activity will be measured as described in the SoA (Table 1). Weekly assessments, starting at Week 24 through Week 52, may be required if a participant is receiving steroids or develops an inhibitor. After Week 52, measurement of FVIII activity may increase if warranted by changes in FVIII activity or the presence of inhibitor. If a participant has a FVIII activity level (chromogenic assay) > 150%, FVIII activity measurement will be assessed at least every 3 months until activity levels have stabilized below 150%. If a participant has FVIII administration around the day of a visit when FVIII activity is scheduled, sample collection should be postponed at least 48 or 72 hours based on the FVIII product used (short half-life or long half-life respectively). Measurement of the FVIII activity level, post FVIII infusion, only pertains to scheduled time points, ie, Week 2, Week 4, etc, and not to each time a participant takes FVIII replacement, should FVIII treatment be necessary.

All samples collected from participants for plasma factor FVIII activity levels will be analyzed by central and local laboratories.

- In the central laboratory, FVIII activity will be assessed both by chromogenic and onestage clotting assay.

- In local laboratories, FVIII activity will be assessed by chromogenic or one-stage clotting assay based on local laboratory's usual practice.

8.1.2. Health Outcome Questionnaire

An EQ-5D-5L will be conducted as described in the SoA (Table 1) using a tablet device provided to each site or the participant's mobile phone.

Developed by the EuroQoL Group, the EQ-5D-5L (EuroQoL, 5 dimensions, 5 levels) is considered the premier measure of health status used in the assessment of the Quality Adjusted Life Year. It measures 5 dimensions of health on a 5-point (5L) scale including Mobility, Self-care, Usual activities, Pain/discomfort, and Anxiety/depression.

Also included is a visual analog scale anchored by worst and best imaginable health on a 0 to 100 scale where participants are asked to indicate where on the scale they rate their current health.

8.1.3. Bleeding Episodes and FVIII Concentrates Usage

Bleeding episodes and FVIII concentrate usage will be reviewed as described in the SoA (Table 1). An e-Diary, a handheld device, will be provided to all participants at baseline which will be used until the EOS.

The participants are required to enter any occurrence of hemophilic bleeding episodes (including date, time, location and etiology) and any exogenous FVIII replacement (including date, time, reason and dose) required to treat the bleeds in the e-Diary throughout follow-up regardless of whether or not the participant has resumed prophylactic therapy. If a participant has to resume FVIII prophylaxis treatment, prophylaxis FVIII infusion data will not have to be reported on the e-Diary after Month 24 but they will be recorded in the eCRF. On-demand and preventative infusions will continue to be required to be reported on the e-Diary throughout follow-up.

Follow-up contacts (eg, phone calls) to review safety, bleeding episodes and FVIII concentrates usage should occur every 6 months (±1 month) after the Month 24 visit.

Study sites will review a bleeding e-Diary where the participant records replacement product use, bleeding episodes especially in the target joints, and information on timing and duration. This information is captured in the dataset.

8.2. Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Table 1).

8.2.1. Physical Examinations

A complete physical examination will be performed as described in the SoA (Table 1). Every physical exam will include the vital signs described below. Genital and rectal examination may be excluded if not clinically indicated. Height (cm) will be collected at screening only, and weight (kg) will be collected at screening and baseline only.

8.2.2. Vital Signs

Vital sign measurements will include systolic and diastolic blood pressure (SBP and DBP), HR, RR, and temperature and will be measured during the complete physical examination (Section 8.2.1). Additional measurements will occur on Day 0 and Day 1. Vital signs should be performed per standard of care as long as the same position for each participant is used at every assessment and after a rest period of at least 1 minute.

On the infusion day (Day 0) and Day 1, vital signs will be obtained prior to infusion and then within 5 minutes of the start of the infusion, every 15 (±5) minutes for the length of the infusion, every 15 (±5) minutes until stable (±10 mmHg), then every 30 (±5) minutes until 2 hours post-infusions, then every 4 hours (±10 min) until discharge.

8.2.3. Electrocardiograms

Standard 12-lead ECGs will be performed as described in the SoA (Table 1). If a participant had an ECG conducted within 6 months prior to screening, then it can be used in place of the screening assessment.

An ECG will be obtained after the participant has rested for at least 10 (±5) minutes per standard of care as long as the same position for each participant is used at every assessment.

ECG values of potential clinical concern are listed in Appendix 8.

8.2.4. Clinical Laboratory Tests

See Appendix 2 (Section 10.2) for the list of clinical safety laboratory tests to be performed and the SoA (Table 1) for the timing and frequency. All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

When applicable, laboratory samples are to be collected prior to dosing. Lab samples obtained every 6 months after week 52 do not require a formal visit to the site, except Months 24 and 36.

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

See Appendix 6 for suggested actions and follow-up assessments in the event of potential drug-induced liver injury.

All protocol-required laboratory assessments, as defined in Appendix 2 (Section 10.2), must be conducted in accordance with the laboratory manual and the SoA (Table 1).

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the CRF as an AE, where falling within protocol-specified AE reporting criteria (see Section 8.3.1).

8.2.5. Chest X-Ray

An anterior/posterior or posterior/anterior and lateral chest X-ray will be performed at screening. If a participant had a chest X-ray conducted within 6 months prior to screening, then it can be used in place of the screening assessment.

8.2.6. Liver Elastography

A liver elastography (or any equivalent imaging technology) is required at screening only for participants with a history of hepatitis C or suspicion of chronic liver disease to evaluate for fibrosis. If a participant had a liver elastography conducted within 6 months prior to screening, then it can be used in place of the screening assessment.

8.2.7. Liver Assessment

A liver MRI will be performed for every participant as described in the SoA (Table 1). If a participant had a liver MRI conducted within 6 months prior to screening, then it can be used in place of the screening assessment.

Circulating alpha fetoprotein level will be determined for every participant as described in the SoA (Table 1). Any participant treated with SB-525 (PF-07055480) who has an elevated alpha fetoprotein and MRI mass greater than 2 cm or suspicious for HCC will undergo biopsy using surgical doses of factor VIII replacement, if needed. An MRI of the liver can be replaced by an abdomen CT-scan (or any equivalent imaging technology) – with vascular phases specific for a search of a liver mass – in case the participant has a contraindication to MRI (for example history of hip replacement).

Histopathologic examination and genomic analysis will be performed to determine the origin and nature of the tumor. Detailed instruction on sample collection and tissue preparation for genomic analysis is provided in Appendix 10 (Section 10.10).

An optional liver biopsy substudy is also proposed to participants (Section 8.2.14).

8.2.8. Abdominal Examination

An abdominal examination for hepatomegaly will be performed as described in the SoA (Table 1).

8.2.9. Interim Follow-up Contact

After the completion of Month 24 visit, follow-up contacts (eg, phone calls) every 6 months (±1 month) will be placed in between the on-site visits (at Months 30, 42 and 54) to review general health status, including safety and bleeding episodes as well as any FVIII concentrate usage. If the initial contact attempt is unsuccessful, a minimum of 3 contact attempts must be documented before the interim follow-up can be considered unsuccessful. AEs and concomitant medications should be reviewed and captured as per Sections 8.3 and 6.5, respectively.

Telephone contacts are not required if they fall when a participant is due on site for a blood draw.

8.2.10. Suicidal Ideation and Behavior Risk Monitoring

Not applicable for this study.

8.2.11. Pregnancy Testing

Not applicable for this study.

8.2.12. Rater Qualifications

Not applicable for this study.

8.2.13. Immunogenicity Assessments

Blood samples for immunogenicity assessments will be collected as outlined below.

8.2.13.1. AAV2/6 Antibodies

Samples for neutralizing activity to AAV capsid proteins (neutralizing antibodies) will be collected at screening to determine eligibility for the study. It is recommended to perform the other screening tests only if the AAV2/6 neutralizing activity results are acceptable. The neutralizing activity is to be reassessed if the screening period takes more than 8 weeks.

Samples will also be collected to assess both total and neutralizing antibodies to AAV2/6 over time, as displayed in the SoA (Table 1).

8.2.13.2. FVIII Inhibitor Titer

FVIII inhibitor levels will be measured as displayed in the SoA (Table 1). Weekly assessments (through Week 52) may be required if a participant is receiving steroids or develops an inhibitor. After Week 52, measurement of FVIII inhibitor titer may increase if warranted by changes in FVIII activity or the presence of inhibitor.

8.2.13.3. Interferon-Gamma ELISPOT

Samples for Interferon-gamma ELISPOT will be collected to detect a proliferation of capsid-specific T cells at baseline and Week 6 (±3 days). Additional samples will be collected if there is a 50% drop in Factor VIII expression and/or prior to initiation of a corticosteroid treatment if possible.

8.2.14. Optional Liver Biopsy

An optional liver biopsy can be performed (in participants who consent to do so and as per investigator's judgement) during the postinfusion period until year 5 i.e, the end of the study. The procedure may be repeated once, later in the study, to assess evolution over time (in participants who consent to do so and as per investigator's judgement).

This substudy may be proposed to any participant, unless there is a condition that, in the opinion of the investigator or a hepatologist or radiologist, would make liver biopsy contraindicated.

The exploratory objectives of the substudy are to evaluate vector integration in the liver, the histopathology of the liver tissue and to assess the expression of protein and/or RNA levels of FVIII and other biomarkers of interest in the liver (depending on collected material). A biopsy will be made upon investigator's decision; it can be performed at any time postinfusion to assess liver health, integration and FVIII in the liver, but could also be triggered by sustained elevated FVIII activity levels, by a significant FVIII activity decline, by a sustained ALT elevation > ULN or to assess the long-term gene therapy effects on the liver.

Any participant who consents to the procedure will have a liver biopsy via either transjugular or percutaneous (ultrasound-guided) route, according to the standard procedures of the institution. At least 2 tissue cores will be harvested and additional details for collecting and handling the biopsy specimens are provided in the Laboratory Manual.

FVIII activity levels should be assessed within 7 days before the biopsy and on the day of the biopsy, prior to the procedure. It is recommended that participants have a FVIII activity level of ≥ 50% (or higher, depending on local guidelines and/or investigator discretion) when doing the procedure. As needed, participants may be treated with additional exogenous FVIII replacement products in order to increase their FVIII activity levels to an appropriate level, under the supervision/instruction of the investigator, to ensure the safety of the participant during the procedure.

Participants consenting to the optional liver biopsy are recommended to undergo pre-biopsy assessments such as:

At least 28 days before the procedure:

- Physical examination
- Central laboratory safety panels (hematology and clinical chemistry, including LFTs) and hemostasis parameters
- Liver ultrasound (fasting recommended at least 8 hours prior to ultrasound)
- FibroScan or Fibrotest

At least 7 days before the procedure:

- Central and local FVIII activity level assessments
- Pre-biopsy consultation (with hepatologist and/or radiologist)

On the day of the biopsy before the procedure:

- Participants will be required to observe an 8-hour fasting period before the procedure.
- Brief physical examination
- Central and local laboratory safety panels (hematology and clinical chemistry, including LFTs) and hemostasis parameters
- Central and local FVIII activity level assessments
- As needed, participants may be treated with additional exogenous FVIII replacement products to increase their FVIII activity levels to an appropriate level.

The follow-up care should be done according to the local standard of care. If only a small amount of tissue (< 2 cm) is obtained at the time of the biopsy, the participant may be asked to consent for a second pass. In this case, the original < 2 cm sample should still be retained and handled according to the instructions for handling biopsy specimens in the Laboratory Manual.

Following completion of the biopsy, the participant should remain under observation in the hospital according to the local procedure. Overnight post-procedure observation may be done at the investigator's discretion and/or according to local guidelines.

Whenever applicable, any finding related to the optional biopsy should be further assessed and followed as clinically appropriate to manage the participant's medical care. A hepatologist and/or other specialist clinicians should be consulted if required. Additional liver ultrasound and/or FibroScan may be considered at the discretion of the investigator and/or hepatologist.

8.3. Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible to pursue and obtain adequate information both to determine the outcome and to assess whether the event meets the criteria for classification as an SAE or caused the participant to discontinue the study intervention (see Section 7.1).

Each participant will be questioned about the occurrence of AEs in a nonleading manner.

In addition, the investigator may be requested by Pfizer Safety to obtain specific follow-up information in an expedited fashion.

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each participant begins from the time the participant provides informed consent, which is obtained before the participant's participation in the study (ie, before undergoing any study-related procedure and/or receiving study intervention), through and including to the last visit (completion at 60 months [±1 months]) or at ETV for participants who discontinue.

However, from Month 36 through to the end of study (Month 60), only the following AEs will be actively collected:

- SAEs
- Non-serious AEs determined to be related to study intervention by the investigator or where causality is unknown.

Follow-up by the investigator continues throughout and after the active collection period and until the AE or SAE or its sequelae resolve or stabilize at a level acceptable to the investigator and Pfizer concurs with that assessment.

For participants who are screen failures, the active collection period ends when screen failure status is determined.

If the participant withdraws from the study and also withdraws consent for the collection of future information, the active collection period ends when consent is withdrawn.

If a participant definitively discontinues or temporarily discontinues study intervention because of an AE or SAE, the AE or SAE must be recorded on the CRF and the SAE reported using the CT SAE Report Form.

Investigators are not obligated to actively seek AEs or SAEs after the participant has concluded study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.1.1. Reporting SAEs to Pfizer

All SAEs, occurring in a participant during the active collection period as described in Section 8.3.1 are reported to Pfizer Safety on the CT SAE Report Form immediately upon awareness and under no circumstance should this exceed 24 hours, as indicated in Appendix 3 (Section 10.3). The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

8.3.1.2. Recording Nonserious AEs and SAEs on the CRF

All nonserious AEs and SAEs occurring in a participant during the active collection period as described in Section 8.3.1 are recorded on the CRF. AEs and SAEs that begin after obtaining

informed consent but before the start of study intervention will be recorded on the Medical History/Current Medical Conditions section of the CRF, not the AE section. AEs and SAEs that begin after the start of study intervention are recorded on the AE section of the CRF.

The investigator is to record on the CRF all directly observed and all spontaneously reported AEs and SAEs reported by the participant.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 3 (Section 10.3).

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. For each event, the investigator must pursue and obtain adequate information until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3).

In general, follow-up information will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Any information relevant to the event, such as concomitant medications and illnesses, must be provided. In the case of a participant death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer Safety.

Further information on follow-up procedures is given in Appendix 3 (Section 10.3).

8.3.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/ Independent Ethics Committees (IECs), and investigators.

Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives SUSARs or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the SRSD(s) for the study and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5. Exposure During Pregnancy or Breastfeeding, and Occupational Exposure

Pregnancies of a partner should be recorded using the Pregnancy Initial Report Form. Pregnancies should be followed until final outcome is known. All follow-up for a pregnancy should be submitted on a Pregnancy Follow-up Report Form.

8.3.5.1. Exposure During Pregnancy (EDP)

- Details of all pregnancies in female partners of male participants will be collected after the start of study intervention and until end of study participation.
- If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4 (Section 10.4).
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.3.5.2. Exposure During Breastfeeding

Not Applicable for this study.

8.3.5.3. Occupational Exposure

An occupational exposure occurs when a person receives unplanned direct contact with the study intervention, which may or may not lead to the occurrence of an AE. Such persons may include healthcare providers, family members, and other roles that are involved in the trial participant's care.

The investigator must report occupational exposure to Pfizer Safety within 24 hours of the investigator's awareness, regardless of whether there is an associated SAE. The information must be reported using the CT SAE Report Form. Since the information does not pertain to a participant enrolled in the study, the information is not recorded on a CRF; however, a copy of the completed CT SAE Report Form is maintained in the investigator site file.

8.3.6. Cardiovascular and Death Events

Not applicable for this study.

8.3.7. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

Bleeding episodes as part of the natural history of severe hemophilia are not reported as AEs.

8.3.7.1. Lack of Efficacy

Lack of efficacy is reportable to Pfizer Safety only if associated with an SAE.

8.3.8. Medication Errors

Medication errors may result from the administration or consumption of the study intervention by the wrong participant, or at the wrong time, or at the wrong dosage strength.

Exposures to the study intervention under study may occur in clinical trial settings, such as medication errors.

Safety Event	Recorded on the CRF	Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

Medication errors include:

- Medication errors involving participant exposure to the investigational product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating participant.

Such medication errors occurring to a study participant are to be captured on the medication error page of the CRF, which is a specific version of the AE page.

In the event of a medication dosing error, the sponsor should be notified immediately.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is recorded on the medication error page of the CRF and, if applicable, any associated AE(s), serious and non-serious, are recorded on an AE page of the CRF.

Medication errors should be reported to Pfizer Safety within 24 hours on a CT SAE Report Form only when associated with an SAE.

8.4. Treatment of Overdose

For this study, any dose of SB-525 (PF-07055480) greater than the intended dose for the participant will be considered an overdose.

Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator/treating physician should:

1. Contact the medical monitor immediately.
2. Closely monitor the participant for any AEs/SAEs and laboratory abnormalities.
3. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

4. Overdose is reportable to Safety **only when associated with an SAE.**

8.5. Pharmacokinetics

8.5.1. AAV2/6 Vector Shedding

AAV2/6 genome in plasma, saliva, urine, stool, and semen by polymerase chain reaction (PCR) will be assessed as described in the SoA (Table 1). On Day 0, vector genome titer should be obtained in plasma only at 12 hours from the start of the infusion ±1 hour.

After Week 12, collection will only continue until three consecutive negative samples are obtained on a sample-type basis (Weeks 16, 20, 24, 28, 32, 36, 40, 44, 48, and 52 [±1 week] and every 1 month after Week 52 to Month 60 [±1 month]). For example, after urine sample results are negative 3 consecutive times, the urine sample will stop being collected while the other samples may continue to be collected if they continue to be positive.

8.6. Pharmacodynamics

Blood samples will be drawn to further explore the study drug mechanism of action and immune responses. These may include, but are not limited to, sEGFR, Gal3BP, von Willebrand factor (vWF), FVIII antigen levels, IL6 and CRP as specified in the SoA (Table 1).

At each visit where vWF is planned, one plasma sample will also be banked to allow for any additional exploratory assessments.

In addition, weekly assessments of FVIII antigen may be required if a participant is receiving steroids or develops an inhibitor or if warranted by changes in FVIII activity.

Blood samples will be collected and processed according to the instructions provided in the laboratory manual.

8.7. Genetics

FVIII gene sequencing will be performed at screening but only for participants with no prior FVIII sequencing.

A blood sample for DNA isolation will be collected. In the event of DNA extraction failure, a replacement genetic blood sample may be requested from the participant.

See Appendix 5 for information regarding genetic research. Details on processes for collection and shipment and destruction of these samples can be found in the Laboratory Manual.

8.7.1. Specified Genetics

Genetics (specified analyses) are not evaluated in this study.

8.8. Biomarkers

Biomarkers are not evaluated in this study.

8.9. Health Economics

Health economics/medical resource utilization and health economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

Detailed methodology for summary and statistical analyses of the data collected in this study is outlined here and further detailed in a statistical analysis plan (SAP), which will be maintained by the sponsor. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

9.1. Estimands and Statistical Hypotheses

There are no statistical hypotheses and estimands needed for this study.

9.2. Sample Size Determination

This study will enroll approximately 20 participants. Participants who do not complete at least 12 months of the study may be replaced, except participants who experience toxicity due to AEs considered related to study drug and discontinue the study for that reason. These latter participants will not be replaced. In addition, participants who experience transaminitis resulting in loss of efficacy prior to week 4 will not be used to determine dose cohort assignment for subsequent participants. As this is an adaptive design study, the number of participants to be treated at any given dose level will be determined by the available efficacy and safety data in proceeding participants and will not be based on statistical calculations.

9.3. Analysis Set

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF and meet all inclusion/exclusion criteria.
Safety	All participants enrolled in this study who receive any portion of study drug and have at least one post-baseline safety assessment will be included in the safety population. All participants who enrolled in the study and received the study intervention.
Evaluable	All participants enrolled in the study and take the full dose of study intervention and no significant interruption of efficacy measurement.

9.4. Statistical Analyses

All analyses of safety will be performed on the safety population, and participants will be analyzed according to the actual treatment they received. Efficacy analyses will be performed using descriptive methods. No formal statistical analysis of study data is planned.

The SAP will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

9.4.1. Primary Endpoints Analyses

Safety analyses will be performed on data from all participants in the Safety Population.

The incidence of AEs and SAE will be tabulated by dose and overall across dose groups. The rate of occurrence for each adverse event will be summarized by body system, severity, and relation to the administration of PF-07055480. All serious adverse events will be summarized separately.

Additional descriptive analyses to support the primary endpoint will include changes from baseline in clinical laboratory variables (including FIX inhibitor and laboratory parameters for thrombotic potential), vital signs, and vector shedding analysis.

The time course profile of FVIII activity will be determined by measuring changes in circulating FVIII activity.

9.4.2. Secondary Endpoints Analyses

The clinical impact on hemophilia after dosing with SB-525 (PF-07055480) will be determined from the summarization of the change from baseline in the use of FVIII

replacement therapy and the frequency and severity of bleeding episodes. Changes from baseline in the EQ-5D-5L will also be summarized.

The immune response to SB-525 (PF-07055480) will be evaluated from the summarization of FVIII inhibitor levels. Summarization of vector shedding data will include vector DNA detection by PCR in plasma, saliva, urine, stool, and semen.

9.4.3. Exploratory Endpoints Analyses

The impact of potentially relevant circulating factors on FVIII activity will be assessed by summarization of vWF, CRP, IL6, and FVIII antigen. Immune response will be studied by measuring neutralizing activity and antibodies to AAV2/6, as well as Interferon-gamma ELISPOT.

Change from the preceding year values may be calculated for selected clinical and laboratory parameters. Shift-tables (change-from-baseline relative to the normal range) may be constructed for selected laboratory parameters. Participants will continue to be followed for years 2 through 5 to gain additional data on safety and durability of effect.

Details for additional exploratory endpoint analyses (eg. optional liver biopsy) will be provided in the SAP.

9.5. Interim Analyses

An interim analysis of safety and efficacy data will be conducted at 1 year after the last enrolled participant receives SB-525 (PF-07055480) infusion. As this is an open-label study, the sponsor may conduct unblinded reviews of the data during the course of the study for the purpose of safety assessment, facilitating dose-escalation decisions, facilitating pharmacokinetic (PK)/pharmacodynamic (PD) modeling, and/or to support clinical development.

9.6. Data Monitoring Committee

This study will use an external data monitoring committee (DMC). The DMC is independent of the study team and includes only external members. The DMC will convene approximately every 6 months until study completion to monitor the safety and efficacy of the participants. Ad-hoc meetings will be organized as needed (eg, to assess events of special interest or after receipt of a SUSAR).

The DMC will be responsible for ongoing monitoring of the efficacy and safety of participants in the study according to the charter. The recommendations made by the DMC to alter the conduct of the study will be forwarded to the appropriate Pfizer personnel for final decision. Pfizer will forward such decisions, which may include summaries of aggregate analyses of endpoint events and of safety data that are not endpoints, to regulatory authorities, as appropriate.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations, including applicable privacy laws.

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be reviewed and approved by the sponsor and submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.1.1. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

In the event of any prohibition or restriction imposed (ie, clinical hold) by an applicable regulatory authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the study intervention, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study participants against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

The investigator or his representative will explain the nature of the study to the participant and answer all questions regarding the study. The participant should be given sufficient time and opportunity to ask questions and to decide whether or not to participate in the trial.

Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, privacy and data protection requirements, where applicable, and the IRB/EC or study center.

The investigator must ensure that each study participant is fully informed about the nature and objectives of the study, the sharing of data related to the study, and possible risks associated with participation, including the risks associated with the processing of the participant's personal data.

The participant must be informed that his personal study related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/EC members, and by inspectors from regulatory authorities.

The investigator further must ensure that each study participant is fully informed about his right to access and correct his or her personal data and to withdraw consent for the processing of his or her personal data.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICD.

Participants must be reconsented to the most current version of the IRB/EC-approved ICD(s) during their participation in the study as required per local regulation.

A copy of the ICD(s) must be provided to the participant.

Participants who are rescreened are required to sign a new ICD.

10.1.4. Data Protection

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of participant data.

Participants' personal data will be stored at the study site in encrypted electronic and/or paper form and will be password protected or secured in a locked room to ensure that only authorized study staff have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site will be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of participants with regard to the processing of personal data, participants will be assigned a single, participant specific numerical code. Any participant records or data sets that are transferred to the sponsor will contain the numerical code; participant names will not be transferred. All other identifiable data transferred to the sponsor will be identified by this single, participant specific code. The study site will maintain a confidential list of participants who participated in the study, linking each participant's numerical code to his or her actual identity and medical record identification. In case of data transfer, the sponsor will protect the confidentiality of participants' personal data consistent with the clinical study agreement and applicable privacy laws.

Information technology systems used to collect, process, and store study-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorized disclosure or access.

The sponsor maintains standard operating procedures on how to respond in the event of unauthorized access, use, or disclosure of sponsor information or systems.

10.1.5. Dissemination of Clinical Study Data

Data or results generated in the performance of the study may be used by sponsor for any purpose, including in registration documents for regulatory authorities in the US or abroad, or for public dissemination in the form of papers, abstracts, posters, or other informational materials to be presented at scientific meetings, or published in professional journals, or as a part of an academic thesis. Sponsor shall have the right to first publication of the data or results of the study, which is intended to be a joint, multi-center publication of the study results made by sponsor in conjunction with the investigators from all appropriate investigational sites contributing data, analysis and comments. Authorship of multi-center publications resulting from this study will be based on customary standards for attribution of authorship taking into consideration factors such as significance of contribution to the design of the study, analysis and interpretation of the data, and critical review of the publication. Subsequent to the first publication of the study results by sponsor, the investigator may publish the site-specific data or results. If the investigator wishes to publish the investigator's site specific data or results, a copy of such proposed publications, papers,

abstracts, or other written materials or an outline of any proposed oral presentations, shall be submitted to sponsor for review at least 60 days (or other number of days agreed to in the applicable clinical trial agreement governing the conduct of the study at the investigator's site) prior to submission of such written materials for publication, or any proposed oral presentation. Sponsor shall have the right to review and comment on such written material or outline, and to confirm the accuracy of the data described therein by comparison with that collected during the course of this study. In addition, sponsor may require investigator to, and investigator will, remove specifically identified confidential information of sponsor (other than the data or results of the study) and/or delay the proposed publication for an additional 60 days (or other number of days agreed to in the applicable clinical trial agreement governing the conduct of the study at the investigator's site) to enable sponsor to file patent applications.

Pfizer fulfills its commitment to publicly disclose clinical study results through posting the results of studies on www.clinicaltrials.gov (ClinicalTrials.gov), the EudraCT/CTIS, and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations. In addition, Pfizer reports study results outside of the requirements of local laws/regulations pursuant to its SOPs.

In all cases, study results are reported by Pfizer in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the study or the country in which the study was conducted.

www.clinicaltrials.gov

Pfizer posts clinical trial US Basic results on www.clinicaltrials.gov for Pfizer-sponsored interventional studies (conducted in participants) that evaluate the safety and/or efficacy of a product, regardless of the geographical location in which the study is conducted. These results are submitted for posting in accordance with the format and timelines set forth by US law.

EudraCT/CTIS

Pfizer posts clinical trial results on EudraCT/CTIS for all Pfizer-sponsored interventional studies in accordance with the format and timelines set forth by EU requirements.

www.pfizer.com

Pfizer posts CSR synopses and plain-language study results summaries on www.pfizer.com for Pfizer-sponsored interventional studies at the same time the corresponding study results are posted to www.clinicaltrials.gov. CSR synopses will have personally identifiable information anonymized.

Documents within marketing applications

Pfizer complies with applicable local laws/regulations to publish clinical documents included in marketing applications. Clinical documents include summary documents and CSRs

including the protocol and protocol amendments, sample CRFs, and SAPs. Clinical documents will have personally identifiable information anonymized.

Data Sharing

Pfizer provides researchers secure access to patient-level data or full CSRs for the purposes of "bona-fide scientific research" that contributes to the scientific understanding of the disease, target, or compound class. Pfizer will make available data from these trials 18 months after study completion. Patient-level data will be anonymized in accordance with applicable privacy laws and regulations. CSRs will have personally identifiable information redacted.

Data requests are considered from qualified researchers with the appropriate competencies to perform the proposed analyses. Research teams must include a biostatistician. Data will not be provided to applicants with significant conflicts of interest, including individuals requesting access for commercial/competitive or legal purposes.

10.1.6. Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF (eCRF) unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

Guidance on completion of CRFs will be provided in the CRF Completion Requirements document.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must ensure that the CRFs are securely stored at the study site in encrypted electronic and/or paper form and are password protected or secured in a locked room to prevent access by unauthorized third parties.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source records and documents. This verification may also occur after study completion. It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Study Monitoring Plan.

The sponsor or designee is responsible for the data management of this study including quality checking of the data.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor. The investigator must ensure that the records continue to be stored securely for so long as they are maintained.

When participant data are to be deleted, the investigator will ensure that all copies of such data are promptly and irrevocably deleted from all systems.

The investigator(s) will notify sponsor or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with sponsor or its agents to prepare the investigator site for the inspection and will allow sponsor or its agent, whenever feasible, to be present during the inspection. The investigator site and investigator will promptly resolve any discrepancies that are identified between the study data and the participant's medical records. The investigator will promptly provide copies of the inspection findings to sponsor or its agent. Before response submission to the regulatory authorities, the investigator will provide sponsor or its agents with an opportunity to review and comment on responses to any such findings.

10.1.7. Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes a source document can be found in the data review plan.

Description of the use of computerized system is documented in the data management plan.

Study Documentation

The investigator and study staff are responsible for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be

suitable for inspection by the sponsor or the FDA at any time and should consist of the following elements:

a) Participant files containing the completed medical records, supporting source documentation, eCRFs and the IRB approved Informed Consent signed by participants.

b) Study files containing all versions of the IRB approved protocol with all amendments, IRB approved informed consent forms, copies of all pre-study documentation, Form FDA 1572 and all correspondence to and from the IRB and the sponsor.

c) The investigator should maintain a list of appropriately qualified persons who are delegated to perform significant study-related studies. In addition, the investigator should maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on the source documents and electronic case report forms.

Record Retention

According to 21 CFR 312.62(c), the investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated. If no application is to be filed or if the application is not approved for such indication, the investigator shall retain these records until 2 years after the investigation is discontinued and the FDA is notified. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with sponsor. It is the responsibility of sponsor to inform the investigator as to when these documents no longer need to be retained.

10.1.8. Study and Site Start and Closure

The study start date is the date of the first participant's first visit.

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor, including (but not limited to) regulatory authority decision, change in opinion of the IRB/EC, or change in benefit-risk assessment. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time upon notification to the sponsor or designee/CRO if requested to do so by the responsible IRB/EC or if such termination is required to protect the health of study participants.

Reasons for the early closure of a study site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/EC or local health authorities, the sponsor's procedures, or GCP guidelines;

- Inadequate recruitment of participants by the investigator;
- Discontinuation of further study intervention development.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the ECs/IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

Study termination is also provided for in the clinical study agreement. If there is any conflict between the contract and this protocol, the contract will control as to termination rights.

10.1.9. Publication Policy

For multicenter trials, the primary publication will be a joint publication developed by the investigator and Pfizer reporting the primary endpoint(s) of the study covering all study sites. The investigator agrees to refer to the primary publication in any subsequent publications. Pfizer will not provide any financial compensation for the investigator's participation in the preparation of the primary congress abstract, poster, presentation, or primary manuscript for the study.

Investigators are free to publish individual center results that they deem to be clinically meaningful after publication of the overall results of the study or 12 months after primary completion date or study completion at all sites, whichever occurs first, subject to the other requirements described in this section.

The investigator will provide Pfizer an opportunity to review any proposed publication or any other type of disclosure of the study results (collectively, "publication") before it is submitted or otherwise disclosed and will submit all publications to Pfizer 30 days before submission. If any patent action is required to protect intellectual property rights, the investigator agrees to delay the disclosure for a period not to exceed an additional 60 days upon request from Pfizer. This allows Pfizer to protect proprietary information and to provide comments, and the investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study intervention or Pfizer related information necessary for the appropriate scientific presentation or understanding of the study results. For joint publications, should there be disagreement regarding interpretation and/or presentation of specific analysis results, resolution of, and responsibility for, such disagreements will be the collective responsibility of all authors of the publication.

For all publications relating to the study, the investigator and Pfizer will comply with recognized ethical standards concerning publications and authorship, including those established by the International Committee of Medical Journal Editors. The investigator will disclose any relationship with Pfizer and any relevant potential conflicts of interest, including any financial or personal relationship with Pfizer, in any publications. All authors will have access to the relevant statistical tables, figures, and reports (in their original format) required to develop the publication.

10.1.10. Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical personnel for the study is documented in the study contact list located in the ISF.

To facilitate access to appropriately qualified medical personnel on study-related medical questions or problems, participants are provided with a contact card. The contact card contains, at a minimum, protocol and study intervention identifiers, participant study numbers, contact information for the investigator site, and contact details for a contact center in the event that the investigator site staff cannot be reached to provide advice on a medical question or problem originating from another healthcare professional not involved in the participant's participation in the study. The contact number can also be used by investigator staff if they are seeking advice on medical questions or problems; however, it should be used only in the event that the established communication pathways between the investigator site and the study team are not available. It is therefore intended to augment, but not replace, the established communication pathways between the investigator site and the study team for advice on medical questions or problems that may arise during the study. For sites other than a Pfizer CRU, the contact number is not intended for use by the participant directly, and if a participant calls that number, he or she will be directed back to the investigator site.

10.2. Appendix 2: Clinical Laboratory Tests

The following safety and efficacy laboratory tests will be performed at times defined in the SoA (Table 1).

Additional laboratory results may be reported on these samples as a result of the method of analysis or the type of analyzer used by the clinical laboratory, or as derived from calculated values. These additional tests would not require additional collection of blood. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

The tests detailed below will be performed by the Central Laboratory (CL) unless otherwise indicated Local Laboratory (LL).

Hematology (CBC with Differential and Platelet Count) - Safety

Platelet Count	RBC Indices:	Automated WBC Differential (absolute and percent):
RBC Count	MCV	Neutrophils
WBC Count (absolute)	MCH	Lymphocytes
Hemoglobin	MCHC	Monocytes
Hematocrit		Eosinophils
Reticulocytes		Basophils

Clinical Chemistry - Safety

Serum Chemistry	**Liver Function Tests (CL and LL)**
Blood Urea Nitrogen	AST (SGOT)
Creatinine	ALT (SGPT)
Glucose	GGT
Sodium	Alkaline phosphatase
Potassium	Total and direct bilirubin
Chloride	
Total CO_2	
Calcium, Magnesium, Phosphate	
Uric Acid	
Albumin	
Total Protein	
Lactate dehydrogenase	

Factor VIII activity (CL and LL)

Central FVIII activity assessed by both chromogenic and one-stage assays
Local FVIII activity assessed by chromogenic or one-stage assays

Immunogenicity

Anti-AAV2/6 antibodies (neutralizing and total)
FVIII inhibitor testing
T-cell responses specific to AAV2/6 and FVIII by ELISPOT assay

Vector Shedding

Detection of AAV2/6 vector by PCR in plasma, saliva, urine, stool and semen

Urinalysis - Safety

Specific gravity
pH, glucose, protein, blood and ketones
Blood or protein if >trace positive
Microscopic exam if macroscopic blood or protein is small (1+), moderate (2+), or large (3+)

Exploratory Parameters

Study drug mechanism of action and immune responses: including but not limited to IL6, CRP, soluble epidermal growth factor receptor (sEGFR), galectin-3-binding protein (Gal3BP), C-reactive protein (CRP)
FVIII antigen
vWF

Other Parameters

HIV RNA viral load and HIV 1/2 antibody assay - screening only
Hepatitis B panel, hepatitis C virus antibody, and HCV RNA viral load – screening only
Coagulation parameters: PT, aPTT, INR
Circulating alpha fetoprotein
FVIII gene sequencing

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events <u>Meeting</u> the AE Definition

• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital sign measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator. Any abnormal laboratory test results that meet any of the conditions below must be recorded as an AE:
 	
  • Is associated with accompanying symptoms.
 	
  • Requires additional diagnostic testing or medical/surgical intervention.
 	
  • Leads to a change in study dosing (outside of any protocol-specified dose adjustments) or discontinuation from the study, significant additional concomitant drug treatment, or other therapy.
 	
• Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition.
 	
• New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
 	
• Signs, symptoms, or the clinical sequelae of a suspected drugdrug interaction.
 	
• Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/selfharming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Death
b. Is life-threatening An AE in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

The term disability means a substantial disruption of a person's ability to conduct normal life functions.

This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording/Reporting and Follow-Up of AE and/or SAE

AE and SAE Recording/Reporting

The table below summarizes the requirements for recording AEs on the CRF and for reporting SAEs on the Clinical Trial (CT) SAE Report Form to Pfizer Safety. These requirements are delineated for 3 types of events: (1) SAEs; (2) non-serious adverse events (AEs); and (3) exposure to the study intervention under study during pregnancy or breastfeeding, and occupational exposure.

It should be noted that the CT SAE Report Form for reporting of SAE information is not the same as the AE page of the CRF. When the same data are collected, the forms must be completed in a consistent manner. AEs should be recorded using concise medical terminology and the same AE term should be used on both the CRF and the CT SAE Report Form for reporting of SAE information.

Safety Event	Recorded on the CRF	Reported on the CT SAE Report Form to Sponsor Safety Within 24 Hours of Awareness
SAE	All	All
Non-serious AE	• Until Month 36: All • After Month 36: only AEs related to the study drug, or AEs with an unknown cause	None
Exposure to the study intervention under study during pregnancy or breastfeeding, and occupational exposure	None	All (And EDP supplemental form for EDP)

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostic reports) related to the event.

The investigator will then record all relevant AE/SAE information in the CRF.

It is not acceptable for the investigator to send photocopies of the participant's medical records to Pfizer Safety in lieu of completion of the CT SAE Report Form/AE/SAE CRF page.

There may be instances when copies of medical records for certain cases are requested by Pfizer Safety. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to Pfizer Safety.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

Intensity will be categorized by toxicity grade according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.

AEs not listed in the CTCAE version 4.03 will be evaluated by using the following criteria:

- Grade 1, Mild: Symptoms causing no or minimal interference with usual social & functional activities;

- Grade 2, Moderate: Symptoms causing greater than minimal interference with usual social & functional activities;
- Grade 3, Severe: Symptoms causing inability to perform usual social & functional activities;
- Grade 4, Potentially Life-threatening: Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death;
- Grade 5: For any AE where the outcome is death.

All Grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as AEs if not associated with a diagnosis already reported on the CRF. A Grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the investigator.

Assessment of Causality

Any AE that does not meet the definition of a suspected AE reaction will be categorized as Not Related.

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to sponsor. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to sponsor.**

- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.
- If the investigator does not know whether or not the study intervention caused the event, then the event will be handled as "related to study intervention" for reporting purposes, as defined by the sponsor. In addition, if the investigator determines that an SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, and report such an assessment in the dedicated section of the CT SAE Report Form and in accordance with the SAE reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide sponsor with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4. Reporting of SAEs

SAE Reporting CT SAE Report Form

- Facsimile transmission of the CT SAE Report Form is the preferred method to transmit this information to Pfizer Safety.
- In circumstances when the facsimile is not working, notification by telephone is acceptable with a copy of the CT SAE Report Form sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the CT SAE Report Form pages within the designated reporting time frames.

10.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

To avoid the transmission of vector-related material and pregnancy, all sexually active participants are required to 1 of the following acceptable forms of contraceptives during the study until at least 3 consecutive post-treatment semen samples are negative for AAV2/6 and for a minimum of 90 days after SB-525 (PF-07055480) administration:

- Double barrier contraceptive including use of a condom plus one other form of contraception used by the participant or partner (eg, vasectomy, intrauterine device [IUD], birth control pills, and diaphragm with spermicide).
- A condom must be used for any form of sexual activity to prevent the transmission of vector-related material.
- Sexual abstinence.

Pregnancies of a partner should be recorded using the Pregnancy Initial Report Form. Pregnancies should be followed until final outcome is known. All follow-up for a pregnancy should be submitted on a Pregnancy Follow-up Report Form.

10.5. Appendix 5: Genetics

Use/Analysis of DNA

- Genetic variation may impact a participant's response to study intervention, susceptibility to, and severity and progression of disease. Therefore, where local regulations and IRB/IEC allow, a blood sample will be collected for DNA analysis.

- The results of genetic analyses may be reported in the CSR or in a separate study summary, or may be used for internal decision-making without being included in a study report.

- The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.

10.6. Appendix 6: Liver Safety: Suggested Actions and Follow-up Assessments

Potential Cases of Drug Induced Liver Injury

Humans exposed to a drug who show no sign of liver injury (as determined by elevations in transaminases) are termed "tolerators," while those who show transient liver injury, but adapt are termed "adaptors". In some participants, transaminase elevations are a harbinger of a more serious potential outcome. These participants fail to adapt and therefore are "susceptible" to progressive and serious liver injury, commonly referred to as drug-induced liver injury (DILI). Participants who experience a transaminase elevation above 3 × ULN should be monitored more frequently to determine if they are an "adaptor" or are "susceptible".

In the majority of DILI cases, elevations in AST and/or ALT precede total bilirubin (TBili) elevations (>2 × ULN) by several days or weeks. The increase in TBili typically occurs while AST/ALT is/are still elevated above 3 × ULN (ie, AST/ALT and TBili values will be elevated within the same lab sample). In rare instances, by the time TBili elevations are detected, AST/ALT values might have decreased. This occurrence is still regarded as a potential DILI. Therefore, abnormal elevations in either AST **OR** ALT in addition to TBili that meet the criteria outlined below are considered potential DILI (assessed per Hy's law criteria) cases and should always be considered important medical events, even before all other possible causes of liver injury have been excluded.

The threshold of laboratory abnormalities for a potential DILI case depends on the participant's individual baseline values and underlying conditions. Participants who present with the following laboratory abnormalities should be evaluated further as potential DILI (Hy's law) cases to definitively determine the etiology of the abnormal laboratory values:

- Participants with AST/ALT and TBili baseline values within the normal range who subsequently present with AST OR ALT values >3 × ULN AND a TBili value >2 × ULN with no evidence of hemolysis and an alkaline phosphatase value <2 × ULN or not available;

- For participants with baseline AST OR ALT OR TBili values above the ULN, the following threshold values are used in the definition mentioned above, as needed, depending on which values are above the ULN at baseline:

    a. Preexisting AST or ALT baseline values above the normal range: AST or ALT values >2 times the baseline values AND >3 × ULN; or >8 × ULN (whichever is smaller).

    b. Preexisting values of TBili above the normal range: TBili level increased from baseline value by an amount of at least 1 × ULN or if the value reaches >3 × ULN (whichever is smaller).

Rises in AST/ALT and TBili separated by more than a few weeks should be assessed individually based on clinical judgment; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor. The participant should return to the investigator site and be evaluated as soon as possible, preferably within

48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history, and physical assessment.

In addition to repeating measurements of AST and ALT and TBili for suspected cases of Hy's law, additional laboratory tests should include albumin, CK, direct and indirect bilirubin, GGT, PT/INR, total bile acids, and alkaline phosphatase. Consideration should also be given to drawing a separate tube of clotted blood and an anticoagulated tube of blood for further testing, as needed, for further contemporaneous analyses at the time of the recognized initial abnormalities to determine etiology. A detailed history, including relevant information, such as review of ethanol, acetaminophen/paracetamol (either by itself or as a coformulated product in prescription or over-the-counter medications), recreational drug, supplement (herbal) use and consumption, family history, sexual history, travel history, history of contact with a jaundiced person, surgery, blood transfusion, history of liver or allergic disease, and potential occupational exposure to chemicals, should be collected. Further testing for acute hepatitis A, B, C, D, and E infection and liver imaging (eg, biliary tract) and collection of serum samples for acetaminophen/paracetamol drug and/or protein adduct levels may be warranted.

All cases demonstrated on repeat testing as meeting the laboratory criteria of AST/ALT and TBili elevation defined above should be considered potential DILI (Hy's law) cases if no other reason for the LFT abnormalities has yet been found. **Such potential DILI (Hy's law) cases are to be reported as SAEs, irrespective of availability of all the results of the investigations performed to determine etiology of the LFT abnormalities.**

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology.

10.7. Appendix 7: Safety Monitoring and Mitigation Plan

The liver function (AST, ALT, bilirubin, alkaline phosphatase, and GGT) of study participants will be monitored closely throughout the study.

Based on preclinical evidence, SB-525 (PF-07055480) has the potential to deliver effective FVIII activity at low administered AAV doses, thus avoiding the transaminitis observed with other gene therapy products at higher AAV doses. For this reason, routine steroid prophylaxis is not planned for initial doses or any dose levels. This study, however, will closely monitor LFT (at least twice weekly through the second LTF determination during Week 20 following dosing) and FVIII activity (at least weekly for through Week 20) after dosing to assess for any potential transaminitis and/or effects on FVIII activity. Steroid treatment will be initiated (recommended: prednisone 60 mg daily or equivalent) in any participant who experiences an ALT increase >1.5 × baseline level after dosing with SB-525 (PF-07055480). Note that the baseline ALT levels will be determined by the central laboratory with subsequent LFTs (ALT and AST at a minimum) performed by the local laboratories for safety. Additionally, steroid treatment may not be initiated if medically contraindicated.

In the case of ALT elevation requiring steroid, LFTs will continue to be followed twice weekly, and steroids will continue to be dosed until the ALT value has stabilized (3 consecutive values under the ULN with no increase of ALT). At that point, if steroids were taken for more than 3 weeks, a steroid taper, with close laboratory monitoring, can be initiated per the following table:

	Week 1	Week 2	Week 3	Week 4
Prednisone	60 mg/day	30 mg/day	15 mg/day	5 mg/day

The steroid taper can be halted and the dosing reverted at any time to a higher prednisone dose if the 3 consecutive values of ALT are not decreasing to baseline and/or ALT values are above the ULN. If 3 consecutive values are below the ULN but have not fully returned to baseline, the taper can continue after discussion with the medical monitor. Refer to SoA Table (Table 1).

Routine steroid prophylaxis will be considered for all current and future dose levels if ALT elevation is observed in 2 participants above 1.5 × ULN, or, above 5 × ULN in 1 participant. DMC will be contacted or convened appropriately. The regulatory authorities will be notified and will review plans if prophylaxis implementation is planned. Note that while ALT changes from baseline will guide decisions to initiate steroid therapy within an individual, changes above the ULN will guide criteria to convene the DMC or decide future prophylactic steroid recommendations. If warranted, the prophylactic steroid regimen would be defined by the DMC in concert with the sponsor.

10.8. Appendix 8: ECG Findings of Potential Clinical Concern

ECG Findings That <u>May</u> Qualify as Adverse Events

- Marked sinus bradycardia (rate <40 bpm) lasting minutes.
- New PR interval prolongation >280 msec.
- New prolongation of QTcF to >480 msec (absolute) or by ≥60 msec from baseline.
- New-onset atrial flutter or fibrillation, with controlled ventricular response rate: ie, rate <120 bpm.
- New-onset type I second-degree (Wenckebach) AV block of >30 seconds' duration.
- Frequent PVCs, triplets, or short intervals (<30 seconds) of consecutive ventricular complexes.

ECG Findings That <u>May</u> Qualify as Serious Adverse Events

- QTcF prolongation >500 msec.
- New ST-T changes suggestive of myocardial ischemia.
- New onset left bundle branch block (QRS >120 msec).
- New onset right bundle branch block (QRS >120 msec).
- Symptomatic bradycardia.
- Asystole:
  - In awake, symptom free patients in sinus rhythm, with documented periods of asystole ≥3.0 seconds or any escape rate <40 bpm, or with an escape rhythm that is below the AV node;
  - In awake, symptom free patients with atrial fibrillation and bradycardia with 1 or more pauses of at least 5 seconds or longer;
  - Atrial flutter or fibrillation, with rapid ventricular response rate: rapid = rate >120 bpm.
- Sustained supraventricular tachycardia (rate >120 bpm) ("sustained" = short duration with relevant symptoms or lasting >1 minute).
- Ventricular rhythms >30 seconds' duration, including idioventricular rhythm (heart rate <40 bpm), accelerated idioventricular rhythm (HR 40 bpm to <100 bpm), and

monomorphic/polymorphic ventricular tachycardia (HR >100 bpm (such as torsades de pointes)).

- Type II second -degree (Mobitz II) AV block.
- Complete (third degree) heart block.

ECG Findings That Qualify as Serious Adverse Events

- Change in pattern suggestive of new myocardial infarction.
- Sustained ventricular tachyarrhythmias (>30 seconds' duration).
- Second or third degree AV block requiring pacemaker placement.
- Asystolic pauses requiring pacemaker placement.
- Atrial flutter or fibrillation with rapid ventricular response requiring cardioversion.
- Ventricular fibrillation/flutter.
- At the discretion of the investigator, any arrhythmia classified as an adverse experience.

The enumerated list of major events of potential clinical concern are recommended as "alerts" or notifications from the core ECG laboratory to the investigator and Pfizer study team, and not to be considered as all inclusive of what to be reported as AEs/SAEs.

10.9. Appendix 9: Abbreviations

Abbreviation	Definition
AAV	adeno-associated virus
AAV2/6	adeno-associated vector 2/6
AE	adverse event
ALT	alanine transaminase
aPTT	activated partial thromboplastin time
AST	aspartate transaminase
BDD	B-domain deleted
BP	blood pressure
bpm	beat(s) per minute
CBC	complete blood count
cDNA	complementary deoxyribonucleic acid
CIOMS	Council for International Organizations of Medical Sciences
CL	central laboratory
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Corona Virus Disease 2019
CRF	case report form
CRO	Contract Research Organization
CRP	C-reactive protein
CRU	clinical research unit
CSR	clinical study report
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DILI	drug-induced liver injury
dL	deciliter
DLT	dose limiting toxicity
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
ECG	electrocardiogram
eCRF	electronic case report form
EDP	exposure during pregnancy
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EMA	European Medicines Agency
EOS	end of study
EQ-5D-5L	EuroQol, 5 Dimensions, 5 Levels
ETV	Early Termination Visit
EU	European Union
EudraCT	European Clinical Trials Database
F8	Factor 8 gene
FDA	US Food and Drug Administration
FVIII	Factor VIII
FIX	Factor IX clotting protein

Abbreviation	Definition
Gal3BP	Galectin-3-binding protein
GCP	Good clinical practice
GGT	gamma-glutamyl transferase
HCC	Hepatocellular carcinoma
HCV	hepatitis C virus
hF8	human F8 gene
HIV	human immunodeficiency virus
HR	heart rate
ICD	informed consent document (synonymous with ICF)
ICF	informed consent form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IL	interleukin
IMP	Investigational medicinal product
IND	Investigational New Drug
INR	International Normalized Ratio
IP	investigational product
IRB	Institutional Review Board
IRT	interactive response technology
ISF	investigator site file
ITR	inverted terminal repeat
IU	international units
IUD	intrauterine device
IV	intravenous
IVRS/IWRS	Interactive Voice Response System/Interactive Web Response System
LFT	liver function tests
LL	Local Laboratory
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MRI	magnetic resonance imaging
N/A	Not Applicable
NHP	non-human primates
NIMP	noninvestigational medicinal product
NSAIDs	non-steroidal anti-inflammatory drugs
OHR	Office for Human Research
PACL	Protocol Administrative Change Letter
PCR	polymerase chain reaction
PD	pharmacodynamics(s)
PK	pharmacokinetic(s)
PT	prothrombin time
PVC	Polyvinyl chloride
QTcF	corrected QT (Fridericia method)
rAAV	recombinant adeno-associated viral vector
RBC	red blood cell

Abbreviation	Definition
RNA	ribonucleic acid
RR	respiratory rate
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
sEGFR	soluble epidermal growth factor receptor
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SMC	Safety Monitoring Committee
SoA	schedule of activities
SOP	standard operating procedure
SP	signal peptide
SRSD	single reference safety document
SUSAR	suspected unexpected serious adverse reaction
TBili	total bilirubin
ULN	upper limit of normal
US	the United States
Vg	vector genome
vWF	von Willebrand factor
WBC	white blood cell

10.10. Appendix 10: Instruction for Tissue Sample Collection and Tissue Preparation

In the event that certain findings occur in the liver or in other tissues (eg, any participant who has an elevated alpha fetoprotein and MRI mass suspicious for HCC or greater than 2 cm), the investigator may recommend a liver biopsy or tissue collection (using surgical doses of Factor VIII replacement, if needed). Local histopathologic examination and central genomic analysis (eg, integration site sequencing analysis) will be performed.

For liver biopsy substudy, please refer to section 8.2.14.

Also refer to the Laboratory manual for liver biopsy collection and processing.

10.11. Appendix 11: Document History

Document	Version Date	Summary of Changes and Rationale
Amendment 4	05-May-2020	The duration of study was extended to 5 years from a 3-year study, requiring 2 additional study visits (Months 48 and 60), to ensure appropriate long-term follow-up of participants after study intervention in line with the FDA and EMA recommendations for duration of long-term follow-up observations for AAV vectors. As a result of this extension, the protocol was updated to include: o Two additional site visits added at Month 48 and 60. o Additional visits (remote or onsite) for laboratory assessments of liver panel, FVIII activity and FVIII inhibitor titer, every 6 months from Week 52 to Month 60. o A requirement for interim follow-up contacts (phone calls), between yearly visits (at Months 30, 42 and 54), were added in the protocol to review general health status, including safety and bleeding episodes, as well as any FVIII concentrate usage. However, those follow-up phone contacts will not be required if they fall on the same day as a scheduled site visit. o From Month 36 through to the end of study (Month 60), only the following Adverse Events (AEs) and the associated concomitant medications will be actively collected: ▪ Serious Adverse Events (SAEs); ▪ Non-serious AEs determined to be related to study intervention by the investigator or where causality is unknown. The requirement for an additional separate LTFU study was consequently removed from the protocol. The following sections of the protocol have been modified with these changes: • Section 1.1 Synopsis • Section 1.2 Schema • Section 1.3 Schedule of Activities • Section 4.1.3 Early Termination • Section 4.4 End of Study Definition • Section 6.5 Concomitant Therapy

Document	Version Date	Summary of Changes and Rationale
		• Section 8 Assessments and Procedures In response to an EMA request to provide data on additional subjects at the highest dose defined as the pivotal Phase 3 study dose, more than five (5) subjects may be enrolled per cohort, and more specifically in cohort 4. The following section of the protocol has been modified with this change: • Section 4.1.2 Treatment Period The Sangamo Therapeutics Protocol Amendment 03 document was transferred into the new Pfizer Protocol Template, and mandatory and/or recommended Pfizer protocol template language was adopted in all relevant sections as appropriate. This is a global change within the document. The following sections were included as part of the Pfizer protocol template: • Section 4.4 End of Study definition • Section 6.2 Preparation/Handling/Storage/accountability • Section 6.3 Measures to minimize biais: Randomization and blinding • Section 6.4 Study Intervention Compliance • Section 6.6 Dose modification • Section 6.7 Intervention after the End of the Study • Section 7.1 Discontinuation of Study Intervention • Section 7.2.1 Withdrawal of consent • Section 7.3 Lost to Follow Up • Section 8.4 Treatment of Overdose • Section 8.9 Health Economics • Appendix 1 Regulatory, Ethical and Study Oversight Considerations • Appendix 3 Adverse Event definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting • Appendix 5 Genetics • Appendix 6 Liver Safety: Suggested Actions and Follow-up Assessments - Potential Cases of Drug Induced Liver Injury • Appendix 8 ECG findings of Potential Clinical Concerns

Document	Version Date	Summary of Changes and Rationale
		As per Pfizer protocol template, the consequences in case of Study Discontinuation have been clarified. The following section of the protocol has been modified with this change: • Section 7.2 Participant Discontinuation/Withdrawal from the Study The Protocol ID and study intervention nomenclature were also updated to reflect both the legacy and the Pfizer references as noted below. This is a global change within the document. • Protocol ID: SB-525-1603 (Pfizer reference: C3731001) • Study intervention reference: SB-525 (Pfizer reference: PF-07055480) To align with the Phase 3 protocol (C3731003), the dosage will be calculated according to weight (i.e. vg/kg) except in situations where a participant's BMI is > 30 kg/m^2. In addition, the study intervention will be provided in 4.7 ml/vial. The following section of the protocol has been modified with this change: • Section 6.1 Study Intervention(s) Administered Some assessments initially done until W52 only have been added on an annual basis: ECG, EQ-5D-5L, vWF measurement and study drug antibodies (total and neutralizing) testing. To reduce visits and related blood sampling, the frequency of LFT assessments for participants receiving steroids was decreased from twice weekly to once a week, if 3 consecutive results demonstrate stable liver function. The following section of the protocol has been modified with this change: • Section 1.3 Schedule of Activities The laboratory requirements for FVIII activity were updated to clarify that all samples collected from participants for FVIII activity levels will be analyzed by central and local laboratories. Methodologies for sample analysis for FVIII activity levels by the local and central laboratories were also included. The following sections of the protocol have been modified with these changes: • Section 1.3 Schedule of Activities • Section 8.1.1 Factor FVIII Activity

Document	Version Date	Summary of Changes and Rationale
		Frequency of collection of the biological samples to evaluate shedding post-W52 has been clarified; from W52, samples will be collected on a monthly basis until 3 negative samples are obtained. • Section 1.3 Schedule of Activities • Section 8.5.1 AAV2/6 Vector Shedding The primary and exploratory endpoints language was updated to provide clarity around the endpoints. No changes were made to the study objectives. The following sections of the protocol have been modified with this change: • Section 1.1 Synopsis • Section 3 Objectives, Estimands and Endpoints The Preclinical Studies summary text and the Benefit Risk Assessment text was deleted and the Investigators Brochure, which has the most up to date summaries, referenced in the protocol. The following sections of the protocol have been modified with this change: • Section 2.2.3 Preclinical Studies • Section 2.3 Benefit Risk Assessment More information on the EQ-5D-5L questionnaire and on the e-Diary process were added. The following sections of the protocol have been modified • Section 8.1.2 Health Outcome Questionnaire • Section 8.1.3 Bleeding Episodes and FVIII Concentrates usage • Section 1.3 Schedule of Activities The AAV2/6 Antibodies section was updated to differentiate between the samples collected at screening and samples collected after screening. Samples for **neutralizing** activity to AAV capsid proteins (neutralizing antibodies) will be collected at screening to determine eligibility for the study. Samples will also be collected to assess both **total and neutralizing** antibodies to AAV2/6 over time. The following sections of the protocol have been modified with this change: • Section 1.3 Schedule of Activities • Section 8.2.13.1 AAV2/6 Antibodies All references to the Safety Monitoring Committee were updated to external Data Monitoring Committee to align with the Pfizer template language. The following sections of the

Document	Version Date	Summary of Changes and Rationale
		protocol have been modified with this change and any reference to the SMC was replaced by DMC. The following section of the protocol has been modified with this change: • Section 9.6 Data Monitoring Committee Pfizer safety process are followed from the date of the sponsor IND transfer in December 2019. The following sections of the protocol have been modified with this change: • Schedule of Activities: AE assessment begins at time of signing ICD (ie, from the screening visit) • Section 8.3.4 Regulatory Reporting Requirements for SAEs • Section 8.3.5 Exposure During Pregnancy or Breastfeeding, and Occupational Exposure • Appendix 3 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting • Appendix 4 Contraceptive Guidance and Collection of Pregnancy Information In addition, it was clarified that bleeding episodes should not be reported as an Adverse Event. The following section of the protocol was added: • Section 8.3.7 Disease-related Events and/or Disease-related outcomes not qualifying as AEs or SAEs The table of safety and efficacy laboratory tests was updated to include a complete list of samples to be collected and analyzed. No new laboratory tests were added to the table. When applicable, information about methods and frequency of these labs were removed from the in-text section and added to this table or to the SoA. Guidance on lab results review has been added as per Pfizer protocol template. The following sections of the protocol have been modified with this change: • Section 8.2.4 Clinical Laboratory Tests • Appendix 2 Clinical Laboratory Tests The populations for analyses were revised and the Intent-To-Treat population was removed since this population is the same as the safety population. In addition, details on the primary analyses were provided. The following sections of the protocol have been modified with these changes: • Section 9.3 Analysis Set • Section 9.4.1 Primary Endpoints Analyses

Document	Version Date	Summary of Changes and Rationale
		Protocol Administrative Changes and Clarifications related to the COVID-19 situation issued on 27 March 2020 was attached as an Appendix to this protocol amendment. The following section of the protocol was added: • Appendix 11: Protocol Administrative Changes and Clarifications
Amendment 3	31-Jul-2018	Protocol Amendment 3 was modified to add a higher dose of SB-525 based on newly available safety data from other studies and to provide clarity on inclusion and exclusion criteria, and details and timing of study assessments. Key changes were summarized below: • Primary endpoints were updated to include SAEs. • Exploratory endpoints were updated. • Prohibited use of celecoxib was modified to only include high doses (>100 mg/day) in Concomitant Medications section. • Updated with additional dose levels in Dose and Rational for Dose Selection section. • Provided clarification on stopping rules. • Added the Screening and Baseline may be approximately 10 weeks in duration. • Updated the footnotes in the Schedule of Activities table.
Amendment 2	11-Oct-2017	Key changes were summarized below: • Updated exploratory endpoints. • Updated #14 and #15 Exclusion Criteria, to add explanation that an MRI of the liver can be replaced by an abdomen CT-scan. • Administrative changes in several sections to get an earlier input from the Safety Monitoring Committee at every steps of the clinical study. • Clarified requirements for various procedures, including addition of HCV antibody, liver MRI footnote, Physical Exam, Liver Panel footnote, Vital Signs, and added procedure/footnote for Interferon-gamma ELISPOT.
Amendment 1	13-Jan-2017	Protocol was updated to address FDA questions/comments. Key changes were summarized below: • Deleted “recombinant” in the Study Population section. • Removed predictions on FVIII levels, dose decisioning based only on participants’ cumulative data in Study Design section. • Dose escalation will not be permitted simultaneously, the protocol was revised accordingly in Study Design section.

Document	Version Date	Summary of Changes and Rationale
		• Clarified participants will have different lengths of exposure for a given dose level in Dose Escalation Based on Safety section. • Details were added in the Sample Size section.
Original Protocol	16-Nov-2016	Not applicable (N/A)

10.12. Appendix 12: Protocol Administrative Changes and Clarifications

In response to the ongoing global pandemic COVID-19, and the increasing restrictions and concerns on public health, the following changes are being incorporated into the SB-525-1603 (Pfizer reference: C3731001) protocol to clarify alternative solutions to accommodate study procedures during the COVID-19 pandemic.

- In the event that the in-clinic study visit cannot be conducted, mobile phlebotomy should be used for the following assessments at the scheduled or unscheduled visits per the protocol Schedule of Event (Table 1):
  - Vital Signs
  - Blood drawing for the following parameters as applicable:
    - Circulating alpha fetoprotein level
    - CBC with differential and platelet count
    - Serum chemistry
    - Liver panel
    - Coagulation screen (PT, INR, and aPTT)
    - FVIII activity
    - FVIII antigen
    - FVIII inhibitor titer
    - Von Willebrand Factor
    - Interferon-gamma ELISPOT
    - Pharmacodynamic/exploratory tests
  - Plasma, saliva, urine, stool and semen samples collection for AAV2/6 vector shedding if still required.
- The EQ-5D-5L questionnaire can be self-administered by the study participant on his mobile phone if the study-recommended device cannot be used.
- In addition, every effort should be made to follow-up the safety of study participants by phone contact. Video contact can be used if permitted by local regulations. During the phone (or video) contact, the following assessments should be performed:
  - Review and record any new concomitant medications or changes in concomitant medications since last contact;

- Review and record any AEs and SAEs since last contact, including but not limited to COVID-19 related events. The AE and SAE reporting process should be followed per protocol;

- Review bleeding episodes and FVIII concentrates usage reported in the e-diary.

Every effort should be made to ensure that protocol-required tests and procedures are completed as described in the protocol. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator must take all steps necessary to ensure the safety and well-being of the participant. When a protocol-required test cannot be performed, the investigator will document the reason for the missed test and any corrective and preventive actions that he or she has taken to ensure that required processes are adhered to as soon as possible. The study team must be informed of these incidents in a timely manner.

If the sponsor determines that the impact of COVID-19 on protocol visits and procedures and associated timeframe needs to be reported on a case report form (CRF), this will be requested. In case of participant discontinuation, select the most appropriate status for discontinuation in the CRF; if the discontinuation is associated with the current COVID-19 pandemic, enter “COVID-19” in the “Specify Status” field.

11. REFERENCES

1. http://www.hemophilia.org/About-Us/Fast-Facts