NCT #: NCT03734588

TITLE PAGE

PROTOCOL TITLE:	Dose-finding study of SPK-8016 gene therapy in patients with hemophilia A to support future evaluations in individuals with FVIII inhibitors
PROTOCOL NUMBER:	SPK-8016-101
INVESTIGATIONAL PRODUCT/NUMBER:	SPK-8016
PHASE OF DEVELOPMENT	1/2a
INDICATION:	Hemophilia A
SPONSOR:	Spark Therapeutics
SPONSOR ADDRESS:	3737 Market Street, Suite 1300 Philadelphia, PA 19104 USA
REGULATORY AGENCY IDENTIFYING NUMBER(S):	[redacted]
ORIGINAL PROTOCOL DATES:	18 September 2018 (Version 1.0) 29 October 2018 (Version 2.0, Amendment 1)
VERSION DATE:	19 March 2021

This study will be conducted in accordance with the standards of Good Clinical Practice (as defined by the International Conference on Harmonization), the ethical principles that have their origin in the Declaration of Helsinki and all applicable national and local regulations.

SPONSOR SIGNATORY FORM

Protocol Title: Dose-finding study of SPK-8016 gene therapy in patients with hemophilia A to support future evaluations in individuals with FVIII inhibitors

Protocol No: SPK-8016-101

This study protocol was subject to critical review and has been approved by the appropriate protocol reviewers of the Sponsor. The information contained in this protocol is consistent with:

- The current benefit-risk evaluation of the investigational product.
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines and according to applicable local requirements.

The Investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Spark Therapeutics:

[redacted]
Spark Therapeutics

Signature Date (DD-MMM-YYYY)

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for my staff and I to conduct this study as described. I will conduct this study as outlined herein, in accordance with Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonization (ICH), and all applicable local and federal regulatory requirements and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Spark or specified designees. I will discuss the material with them to ensure that they are fully informed about the study.

Institution Name

Principal Investigator (PI) Name/Site Number

Principal Investigator's (PI) Signature Date (DD-MMM-YYYY)

LIST OF PERSONNEL AND ORGANIZATIONS RESPONSIBLE FOR CONDUCT OF THE STUDY

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

PROTOCOL DOCUMENT HISTORY

DOCUMENT HISTORY	
Document	**Date**
Amendment 2	19-Mar-2021
Amendment 1	29-Oct-2018
Original Protocol	18-Sep-2018

A summary of the changes made in this protocol amendment is located in Section 16.

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1 PROTOCOL SUMMARY

Protocol Title:	Dose-finding study of SPK-8016 gene therapy in patients with hemophilia A to support future evaluations in individuals with FVIII inhibitors.
Protocol Number:	SPK-8016-101
Sponsor:	Spark Therapeutics
Development Phase:	Phase 1/2a
Name of Investigational Product:	SPK-8016
Study Indication:	Hemophilia A

Rationale:

The primary aim of hemophilia care is to prevent and treat bleeding due to deficient clotting factors. Both hemophilia A and B can be treated with recombinant factor replacement leading to significant improvement in morbidity and mortality. However, such treatment is extremely costly and is often still associated with clinical complications that include bleeding, particularly bleeding into the joints (hemarthrosis). Moreover, this treatment involves a life-long need for recombinant factor infusions 2 to 3 times per week to maintain minimal therapeutic levels; thus, it is demanding and invasive and may negatively impact an individual's quality of life.

Since genes were initially cloned more than 30 years ago, the curative treatment of hemophilia via gene therapy has been a primary goal. As a novel alternative to the current treatment for hemophilia A, gene therapy could potentially reduce short-term disability and long-term hemophilic arthropathy, reduce incidence of central nervous system (CNS) bleeding, eliminate the need for indwelling intravenous catheters or frequent factor infusion, and improve patients' overall quality of life (QoL) and functional independence (Colvin, 2008). Indeed, recent studies have shown that liver mediated gene therapy with adeno-associated virus (AAV) vectors can safely and consistently achieve sustained clinically meaningful levels of coagulation factor VIII (FVIII) activity and can potentially eliminate spontaneous hemarthrosis. In the Western world,

the hemophilia population with the greatest unmet need are patients who develop inhibitors, or neutralizing antibodies, to FVIII. For these patients, a cornerstone of treatment is the eradication of these neutralizing antibodies after they develop, and data in animal models of hemophilia support the possibility of using gene therapy to carry out this eradication.

SPK-8016 (adeno-associated viral vector with a [redacted]) is being developed by Spark Therapeutics for the treatment of hemophilia A in patients including those with FVIII inhibitors. This initial clinical study (SPK-8016-101) will evaluate the safety, efficacy, and tolerability of SPK-8016 in adult males with clinically severe hemophilia A and no measurable inhibitor against FVIII. The data obtained from this study will inform the design and dose selection for subsequent studies of SPK-8016 in other patient populations (e.g., those with inhibitors).

In this SPK-8016-101 study, 4 participants have already received SPK-8016 at a dose level of $5x10^{11}$ vg/kg. Reactive corticosteroids have been administered to avoid the loss of factor VIII transgene expression. This reactive treatment was initiated ~ 3-6 weeks after vector administration in response to a change in the alanine aminotransferase (ALT), FVIII, or ELISpot. Three of the participants required prolonged tapering of daily oral corticosteroids (total courses of 43-48 weeks prednisone) to avoid and treat late recurrences of apparent hepatocyte-targeted immune response. For 2 of these participants, it was clinically necessary to initiate a steroid sparing regimen (i.e., [redacted]) to aid in the prednisone taper.

Additional investigation will be needed to determine whether there is a short course immunosuppressive regimen that will reliably control immune responses in all patients and allow long-term expression of the donated gene. Going forward, administration of SPK-8016, beginning at the $1x10^{12}$ vg/kg dose, will be explored in 2 cohorts chosen based on prior clinical studies, non-clinical studies, and input from clinical experts in rheumatology, organ transplantation, viral immunology, and bone marrow transplantation. [redacted] prior to vector infusion in an effort to prevent the immune response. In the follow up period, corticosteroids will only be administered if triggered by the clinical observation of increased liver transaminases or decreased circulating FVIII. The ELISpot will not be considered a predictive tool independent of other immune triggers. [redacted]

[redacted] Oral corticosteroids will be used when clinically indicated.

[redacted]

Study Design: SPK-8016-101 is a Phase 1/2a, open-label, non-randomized, dose-escalation study designed to evaluate the safety, efficacy, and tolerability of SPK-8016 in adult males with clinically severe hemophilia A and no measurable inhibitor against FVIII. The data obtained from this study will inform the study design and dose selection for subsequent studies of SPK-8016 in other patient populations (e.g., those with inhibitors).

In this study, approximately 40 evaluable participants will be dosed with a single intravenous infusion of SPK-8016 at one of the following doses (up to n=10 per dose cohort and group):

- Dose group 1 (starting dose): $5x10^{11}$ vg/kg
- Dose group 2 (middle dose): $1x10^{12}$ vg/kg
- Dose group 3 (high dose): $2x10^{12}$ vg/kg

Based on FVIII activity levels observed after any of the planned dose levels, a cohort and dose level may be expanded to up to 10 participants.

Study SPK-8016-101 is composed of 3 study periods: Screening (up to 16 weeks), Dosing (24 hours), and Follow-up Observation (52 weeks). The total duration of the study for an individual participant is up to 68 weeks (including up to 16 weeks of screening). A schematic of the study design is presented in Figure 1.

After completion of Study SPK-8016-101, participants will be consented to enroll in a long-term follow-up (LTFU) study for an additional 4 years

of post-treatment follow-up. If the LTFU study is not enrolling at the time of a participant's planned Week 52 visit, the participant may remain in study SPK-8016-101. The Week 52 procedures are to be performed only at the EOS Visit. The procedures from Week 46, except for collection of immunology samples for enzyme-linked immunospot assay (ELISpot), will be performed at Week 52 and every 12 weeks until the LTFU study is open. At that time, the week 52/EOS visit should occur.

Study Objectives and Endpoints:

Objectives
Primary
• To evaluate the safety and tolerability of SPK-8016. • To evaluate the efficacy as evidenced by prevention of bleeds and level of FVIII expression with SPK-8016.
Secondary
• To evaluate additional parameters associated with SPK-8016 directed FVIII expression and vector shedding. • To characterize the immune response to the vector and transgene product.
Endpoints
Primary

• For safety and tolerability:
  ○ Incidence of adverse events, including clinically significant abnormal laboratory values.
  ○ Occurrence of hepatic transaminase elevation requiring immunosuppression.
• For efficacy:
  ○ Peak and steady-state FVIII activity levels assessed by coagulation clotting assays. In this study, steady-state levels are based on FVIII:C measurements starting 12 weeks post vector administration and without the use of exogenous FVIII products since vector administration.
  ○ Number of bleeding events (spontaneous and traumatic) since vector administration.
  ○ Number of FVIII infusions since vector administration.

Secondary
• Additional kinetic assessments including, but not limited to: o Time to achieve peak and steady-state FVIII activity levels. o Vector shedding of SPK-8016 in bodily fluids. • Incidence of immune responses to AAV capsid protein and BDD-hFVIII transgene.
Exploratory
• Joint assessments • Activities assessments • Quality-of-life assessments • Health-economic parameters • Exploratory inflammatory profiling of plasma and immune function gene expression of Peripheral Blood Mononuclear Cells (PBMCs) after vector administration (ELISpot, and other exploratory biomarkers)

Study Population: Adult males with clinically severe hemophilia A

Inclusion Criteria: Participants must meet all the following eligibility criteria at screening and prior to dosing of SPK-8016 (Day 0) to be eligible for the study:

1. Be able to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (PHI) in accordance with national and local privacy regulations
2. Be male and ≥18 years of age
3. Have clinically severe hemophilia A, defined as:
   a) <1% (<1 IU/dL) endogenous FVIII activity levels as historically documented by a certified laboratory **or** screening data results; OR
   b) 1-2% (1-2 IU/dL) endogenous FVIII activity levels and >10 bleeding events per year (in the last 52 weeks prior to screening); OR
   c) 1-2% (1-2 IU/dL) endogenous FVIII activity levels and on prophylaxis

4. Have had >150 prior exposure days to any recombinant and/or plasma-derived FVIII concentrates or cryoprecipitates based on historical data from medical records/history
5. Have no prior history of hypersensitivity or anaphylaxis associated with any FVIII or IV immunoglobulin administration
6. Have no measurable inhibitor against FVIII at screening (i.e., <0.6 Bethesda Units); no confirmed history of clinically significant FVIII inhibitor ***and*** no clinical signs or symptoms of decreased response to FVIII administration (Note: Family history of inhibitors is not exclusionary, nor is remote documentation (greater than 5 years) of a single measurement of Bethesda titer of >0.6 BU that is not accompanied by clinical evidence of failure to respond to infused FVIII concentrate
7. Have acceptable laboratory values sampled at screening and reviewed prior to Day 0:
   a. Hemoglobin ≥11 g/dL;
   b. Platelets ≥100,000 cells/µL;
   c. Aspartate aminotransferase (AST), ALT, alkaline phosphatase < upper limit of normal (ULN);
   d. Bilirubin ≤1.5x ULN (Bilirubin levels above the laboratory's normal range are acceptable in individuals with a documented history or laboratory evidence of Gilbert's Disease);
   e. Creatinine ≤2.0 mg/dL;
   f. Absolute neutrophil count (ANC) ≥ 2000 per mm^3;
   g. Fibrinogen antigen ≥ 180 mg/dL for [redacted] in Cohort 2
8. Agree to use reliable barrier contraception after the administration of SPK-8016 until notified by the Investigator or designee.

Exclusion Criteria:

Participants who meet any of the following criteria at screening or prior to dosing of SPK-8016 (Day 0) are not eligible for the study:

1. Have active hepatitis B or C. All participants must be screened for both active hepatitis B and C regardless of prior known history.
   a. **Screening for hepatitis B:** All participants must have a single sample collected at screening for each of the following tests: HBsAg (hepatitis B surface antigen), anti-HBc (total Hepatitis B core antibody), and Hepatitis B virus (HBV)-DNA viral assay (nucleic acid test for hepatitis B virus DNA).
      i. A participant is not eligible if either HBsAg is positive or HBV-DNA is positive/detectable.

ii. A participant is eligible if the anti-HBc is positive and both HBsAg and HBV-DNA are negative, as this would be consistent with a prior infection of hepatitis B. Anti-HBc must be obtained in all participants to discriminate between acute infection and possible reactivation of hepatitis B during the trial (e.g., in participants with no prior history of hepatitis B).

b. **Screening for hepatitis C:** All participants, including those who have never been treated or who have completed anti-viral therapy for chronic hepatitis C, must have a single Hepatitis C virus (**HCV)-ribonucleic acid (RNA) load assay (also referred to as a nucleic acid test [NAT] for HCV RNA)** at screening.
   i. A participant is not eligible if his HCV-RNA load assay is positive/detectable.
   ii. A participant treated with anti-viral therapy for chronic hepatitis C must have completed anti-viral therapy at least 6 months prior to screening and must have a negative HCV-RNA at the time of screening.
   iii. A participant with a documented or self-reported history of HCV must have a single negative HCV-RNA at the time of screening.

2. Are currently on antiviral therapy to treat their hepatitis B or C
3. Have significant underlying liver disease. A participant is not eligible with any of the following documented diagnoses, indicative of significant underlying liver disease:

   a. Portal hypertension; *or*

   b. Splenomegaly; *or*

   c. Hepatic encephalopathy.

   Any participant without any of these pre-existing diagnoses must have the following performed at screening:

   a. Serum albumin measurement. A participant is not eligible if the serum albumin level is below the lower limit of normal of the laboratory; ***and***

   b. Diagnostic test for liver fibrosis (e.g., FibroScan, FibroTest/FibroSURE, or AST-to-Platelet Ratio Index [APRI]). A participant is not eligible if any of the following findings, which are indicative of fibrosis ≥stage 3, are present:

      i. FibroScan score >8.3 kPa units; or

      ii. FibroTest/FibroSURE >0.48; or

iii. APRI >1

If more than 1 diagnostic test result is available, then the FibroScan score will be used as the primary consideration for eligibility.

4. Have serological evidence of human immunodeficiency virus (HIV)-1 or HIV-2 with CD4 counts ≤200/mm^3.
    i. Participants who are HIV-positive and stable, with an adequate CD4 count (>200/mm3) and undetectable viral load (<50 gc/mL) measured at screening, and who are on an antiretroviral drug regimen are eligible to enroll
5. Have [redacted] neutralizing antibody titers ≥1:1
6. Have a history of active cancer in the past 6 months, chronic infection, latent or active tuberculosis, uncontrolled immune disorder or other chronic disease that the Investigator and/or Sponsor considers to constitute an unacceptable risk
7. Have been dosed in a previous gene therapy research trial within the last 52 weeks **or** have participated in a clinical study with an investigational drug within the last 12 weeks prior to signing the informed consent
8. Have a history of diverticulitis, diverticulosis requiring antibiotic treatment, or chronic ulcerative lower G.I. disease that might predispose a patient to perforations
9. Have any concurrent clinically significant major disease (such as liver abnormalities, type I diabetes, uncontrolled hypertension, or vertebral compression) or any other condition such as active infections or COVID-19 or any other unspecified reasons that, in the opinion of the Investigator and/or Sponsor, makes the participant unsuitable for participation in the study.
    i. At the time of screening, the Investigator will consider the local geographic and institutional epidemiology of coronavirus disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV 2) (COVID-19) and other infectious pathogens when determining suitability of the participant for participation in the study, including considering the potential clinical relevance of additional screening
10. Have a planned surgical procedure in the next 12 months requiring FVIII prophylactic treatment

11. Are unable or unwilling to comply with the schedule of visits and study assessments described in the clinical protocol.

Study Duration: The total duration of the study is approximately 68 weeks, including up to 16 weeks for screening. The study will include the following phases:

a) Screening period (up to a maximum of 16 weeks);
b) Study Intervention/Dosing day (Day 0);

Follow-up observation period (52 [±2] weeks).

Planned Number of Participants: Up to 40 evaluable participants will be dosed with a single intravenous infusion of SPK-8016.

Number and Location of Study Sites: The study is planned to be conducted at approximately 15 study centers (vector-administration centers and/or follow-up centers) worldwide.

Sequence of Enrollment: Two staggering strategies are employed in this study:

a) The first 2 participants in each dose level will be infused with SPK-8016 at least 6 weeks apart to mitigate acute safety risk; and
b) There will be at least 6 weeks of staggering between each dose level. The Data Monitoring Committee (DMC) will review at least 6 weeks of follow-up data from up to 4 participants who have received SPK-8016 at a given dose level prior to infusing the first participant in the next dose level.
c) Continued enrollment will alternate between Cohort 1 and Cohort 2 beginning at $1x10^{12}$ vg/kg. A stagger of at least 4 weeks will occur between the first and the second participants in Cohort 2 (See Figure 1).

The dose level for Cohorts 1 & 2 will begin at $1x10^{12}$ dose to explore immunomodulation. Cohort 1 will utilize [redacted] begun approximately 48 hours prior to and for 13 weeks following vector infusion. Cohort 2 will utilize [redacted] administered prior to vector infusion.

HIV positive individuals with stable CD4+ count >200 mm3 are eligible provided all other inclusion criteria are met. However, they will not be enrolled as the first 2 participants in Cohort 2.

Criteria for Evaluation:

Safety Evaluation:

Safety will be assessed by physical examination, vital signs, adverse events, measurement of antibodies against FVIII, immune responses against transgene product and/or vector, vector shedding of bodily fluids,

laboratory parameter changes over time, and the use of any immunosuppressive therapy.

The infusion of SPK-8016 to the first 2 participants at each dose level will be staggered by at least 6 weeks to mitigate acute safety risk. Additionally, at least 6 weeks of follow up data from up to 4 participants in a given dose level will undergo review by the DMC prior to infusing the first participant in the next dose level.

Pharmacokinetic Evaluation:

The responses to SPK-8016 will consist of peak and steady-state values of circulating vector-derived FVIII activity levels after SPK-8016 infusion. Additional pharmacokinetic (PK) evaluation of SPK-8016 is carried out by analysis of serum urine, semen, saliva for the presence of vector DNA.

Efficacy Evaluation:

The following information will be collected for efficacy evaluations:

- Vector-derived factor VIII in circulation (FVIII:C) activity levels (including additional peak and steady-state levels)
- FVIII antigen level
- Number of FVIII infusions (prophylaxis and/or on-demand)
  - Number of bleeding episodes (spontaneous and traumatic)
  - Annualized FVIII usage (AFU)
  - Annualized bleeding rate (ABR) (spontaneous and traumatic)
- Joint assessments
- Activities assessments
- Quality-of-life assessments
- Health-economic assessments

Investigational Product Description:

[redacted]

[redacted]

Dose Expansion/ Escalation Plan:

Each dose level is planned to enroll and dose a minimum of 2 participants. The first 2 participants at each dose level will be dosed at least 6 weeks apart to ensure the safety and tolerability of the vector administration.

Dose-Level and Cohort Expansion:

A. ***Dose-Level Expansion*** **-** After 2 participants are dosed at a given dose-level, it may be possible to expand that cohort to up to 10 participants if there is evidence of FVIII:C increases above 5% of normal in either participant by Week 4 post-vector administration. This initial expansion will provide additional information about the variability of responses within the same dose-level.

B. ***New Cohort Expansion*** **–** For Cohorts 1 and 2 of the 5 x 10^{11} vg/kg dose, the first 2 participants in each cohort will be infused at least 6 weeks apart. After 2 participants are dosed in a cohort, an initial expansion of up to 3 participants may occur and, thereafter, an additional expansion for up to a total of 10 participants may occur. The additional expansion may occur in 1 or more cohorts.

Dose Escalation:

The decision to dose escalate will be made by the Sponsor in consultation with the DMC to ensure safety.

Dose escalation to the next dose level may occur under the following scenarios, provided there are no safety concerns after at least 6 weeks of follow-up data have been reviewed by the DMC. Dose escalation through the first 3 dose levels (5x10^{11} vg/kg, 1x10^{12} vg/kg, 2x10^{12} vg/kg) may occur in, but is not restricted to, the following scenarios.

Dose escalation may be considered:

a) If **neither of the first 2 participants** achieve FVIII:C above 5% of normal by Week 6 post vector-administration; or
b) If **at least 2 participants** in a given dose-cohort achieve FVIII:C ≤ 40% of normal by Week 6 post vector-administration; or
c) If **3 participants** in a given dose-cohort achieve 'steady-state' FVIII:C ≤ 50% of normal by Week 6 post vector-administration.

There will be **no** dose escalation if at least 3 participants in any dose level achieve 'steady-state' FVIII:C > 80% of normal. Steady-state levels are based on at least 2 separate FVIII:C measurements, at least 2 weeks apart, starting 8-12 weeks post vector administration while not receiving daily corticosteroid therapy and without use of exogenous FVIII product since vector administration.

After escalation through the first 3 planned dose levels, a fourth dose level may be explored in consultation with the DMC. Additionally, the Sponsor may decide to further expand the starting dose cohort ($5x10^{11}$ vg/kg), the middle dose cohort ($1x10^{12}$ vg/kg), or the high dose ($2x10^{12}$ vg/kg) to better evaluate the safety, efficacy, and variability of response within a given cohort. Any decision to add a fourth dose level or further expand one of the existing protocol-defined dose levels will be made in consultation with the DMC. In consultation with the DMC, further dose exploration may be considered if effective immunomodulation has been demonstrated in any cohort and/or FVIII expression is < 80% of normal 12 weeks following gene transfer in at least 2 participants.

Data and Safety Monitoring Plan:

The independent DMC is composed of at least 3 independent experts in hemophilia or immunology. The independent DMC will be responsible for reviewing safety and efficacy data, as well as other data, as needed, on a periodic basis during the course of the study. The specifics regarding the DMC organization and procedures will be outlined in the DMC Charter.

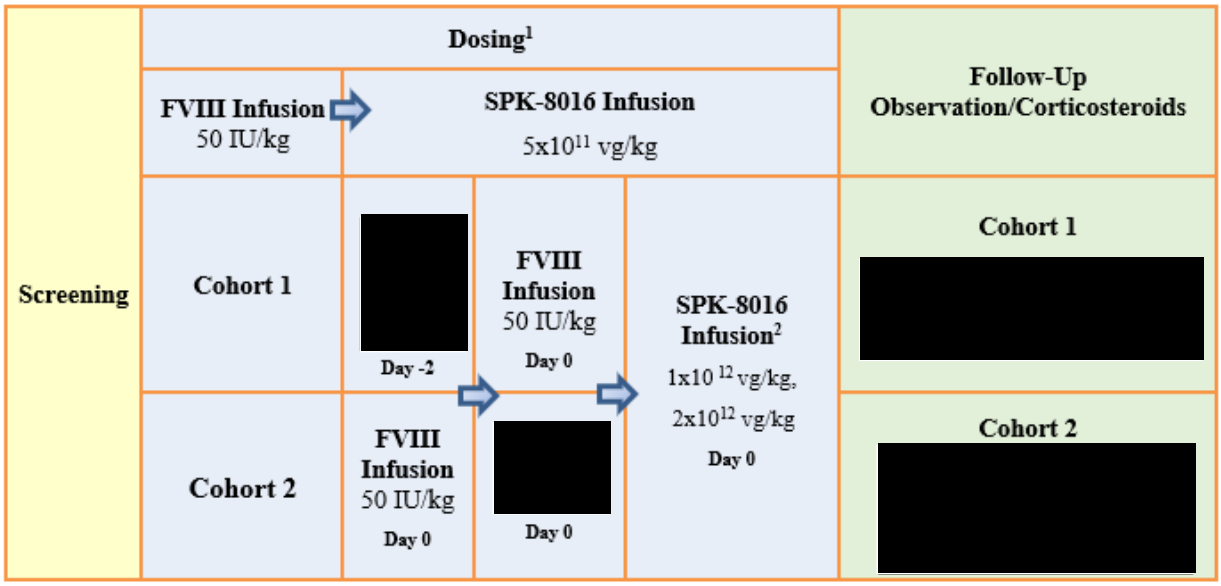
Statistical Methods:

Statistical analyses will be primarily descriptive in nature. Summary statistics will be presented for all safety endpoints and vector-derived FVIII:C activity levels over time after SPK-8016 administration. No formal statistical hypothesis testing will be performed. Various exploratory statistical tests may be applied to data generated from this study to generate hypotheses to be tested in subsequent trials.

In general, descriptive statistics including number of observations, mean, standard deviation, median, minimum, and maximum will be presented for continuous parameters. Categorical parameters will be presented using counts and percentages within each category.

Interim analyses may be performed after at least 2 participants in a given dose cohort complete Week 12.

Figure 1: Study Schema



1. [redacted]
2. The dose level for Cohort 1 and Cohort 2 will begin at $1x10^{12}$ vg/kg. Dose escalation is possible per Section 4.1.1.

Table 1: Schedule of Assessments

	Screen Period	Three to Seven Days prior to Day 0[25]	Day -2	Dosing Period					Follow-Up Observation Period (52 Weeks)																																	
				Day 0					Day 1	Study Days 3-84 (± 2 days)[3, 11]																							Study Weeks 14-52 (± 2 weeks)[2, 14]									
Tests and Assessments	Screen[1] Weeks -16 to -1			FVIII dose	[redacted] Dose	Pre-SPK 8016	SPK8016 Dose	30 (± 2) min, 2 and 5 hrs (±10 mins) from stop of SPK-8016 infusion		3	7	10	14	17	21	24	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	84	14	16	18	22	26	30	34	40	46	52 / EOS[2, 23]
Informed Consent [4]	X																																									
Review Inclusion / Exclusion criteria	X					X																																				
Demographics, Medical and Hemophilia History [5]	X																																									
Genotype, HLA, if not known[5]	X																																									
Target joints, Joint Health Assessment, Hemophilia Joint Health Score	X[6]																																				X					X
Physical Exam, Height, Weight[3,8]	X[7]					X				X													X									X			X		X		X	X		X
Vital Signs	X					X[9]		X[9]	X[9]	X													X									X	X	X	X	X	X	X	X	X	X	X
α-fetoprotein (CL)	X																																									X
Liver ultrasound (*if indicated*)[13]	X[6]																																									X
HBsAg, anti-HBc, HBV-DNA (CL)[10]	X																															X[29]										
HCV-RNA load assay (CL)[10]	X																																									
HIV-1/HIV-2 Ag/Ab (CL)[10]	X																																									
CD4+ count, HIV-1, or HIV-2 viral load (CL)[10]	X																																									
Hematology (CL)[26]	X	X							X	X													X									X	X	X	X		X					X
Clinical Chemistry (CL)	X									X													X									X			X		X					X
Urinalysis (CL) (*using dipstick*)	X																																				X					X
Coagulation – aPTT and FVIII Activity (CL, LL), FVIII antigen (CL)	X					X[17]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fibrinogen Antigen, Thrombin Time, D-Dimer[35] (LL)	X											X				X																										
FVIII inhibitor (CL, LL at Screening)	X																						X									X	X				X			X		X

Tests and Assessments	Screen Period: Screen[1] Weeks -16 to -1	Three to Seven Days prior to Day 0[25]	Day -2	Dosing Period, Day 0: FVIII dose	Day 0: [redacted] Dose	Day 0: Pre-SPK 8016	Day 0: SPK8016 Dose	Day 0: 30 (± 2) min, 2 and 5 hrs (±10 mins) from stop of SPK-8016 infusion	Follow-Up Observation Period (52 Weeks): Day 1	Study Days 3-84 (± 2 days)[3, 11]: 3	7	10	14	17	21	24	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	84	Study Weeks 14-52 (± 2 weeks)[2, 14]: 14	16	18	22	26	30	34	40	46	52 / EOS[2, 23]
VWF Activity & VWF Antigen (CL)	X																																									X
Liver function tests and CRP (LL and CL)[11]	X[11]	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunology (CL)[25]		X				X				X		X		X		X		X		X		X		X		X		X		X		X	X	X	X	X	X	X	X	X	X	X
ECG (*if indicated*)[12]	X																																									
Liver fibrosis diagnostic test[13]	X																																									
Lipid panel, *if indicated* (CL)[14,26]	X																																									X
AAV Neutralizing Antibody (CL)	X									X		X		X		X		X					X									X	X				X			X		X
Vector shedding (CL)[15]	X[6]									X		X		X		<<< *if indicated* >>>																										
Immune Profiling[30,32]		X				X		X[30]	X		X		X		X		X								X									X								
PAX Gene[31,32]		X				X		X[31]	X				X				X								X																	
Spare Plasma (CL)[21]	X								X																							X					X			X		X
Thiopurine Methyltransferase (TPMT) (LL)	X																																									
TB Testing (LL)	X																																									
SARS-CoV2 Testing (LL)[33]	X	X																																	X							
Infusion with FVIII product[16]				X																																						
Infusion with SPK-8016[18]							X																																			
[redacted] regimen, Cohort 2[26,27]					X[27]																																					
[redacted] regimen, Cohort 1[26,28]			X[28]																																							
PK Profile[34]	X																																									
Hem Activities List, Health-economic Assessment[19]	X[6]																						X									X	X				X			X		X
Haem-A-QoL, EQ-5D-5L[19]	X[6]																																				X					X

Tests and Assessments	Screen Period: Screen[1] Weeks -16 to -1	Three to Seven Days prior to Day 0[25]	Day -2	Dosing Period: Day 0: FVIII dose	[redacted] Dose	Pre-SPK 8016	SPK8016 Dose	30 (± 2) min, 2 and 5 hrs (±10 mins) from stop of SPK-8016 infusion	Follow-Up Observation Period (52 Weeks): Day 1	Study Days 3-84 (± 2 days)[3, 11]: 3	7	10	14	17	21	24	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	84	Study Weeks 14-52 (± 2 weeks)[2, 14]: 14	16	18	22	26	30	34	40	46	52 / EOS[2, 23]
Dispense/Review FVIII Infusion Log[20]	X[6]																						X									X	X				X			X		X
Adverse Events	X				<<< throughout the study >>>																																				
Prior and Concomitant Therapies and Procedures[24]	X				<<< throughout the study >>>																																				
Unscheduled visit or safety test[22]					<<< as needed >>>																																				

AAV=adeno-associated virus vector; aPTT=activated partial thromboplastin time; anti-HBc=total hepatitis B core antibody; CD4= cluster of differentiation 4; CL=central laboratory; CRP=c-reactive protein; ECG=electrocardiogram; EOS=end of study; EQ-5D-5L=Euro Quality-of-Life Five Dimensions Questionnaire; FVIII=coagulation factor VIII; Haem-A-QoL=Hemophilia Quality of Life Questionnaire; HBV-DNA=hepatitis B virus-deoxyribonucleic acid; HBsAg= hepatitis B surface antigen; HCV-RNA=hepatitis C virus-ribonucleic acid; HIV-1=human immunodeficiency virus 1; HIV-2= human immunodeficiency virus 2; HIV-1/HIV-2 Ag/Ab=human immunodeficiency virus 1/human immunodeficiency virus 2 antigen/antibody; HLA= human leukocyte antigen; LL=local laboratory; PK=pharmacokinetics; SARS-CoV-2= severe acute respiratory syndrome coronavirus 2; SPK-8016=study drug in this protocol; TB=tuberculosis; [redacted]; TPMT=thiopurine methyltransferase; VWF=von Willebrand factor

1. The Screening period may be up to 16 weeks. If the screening period exceeds 16 weeks, then the participant must repeat all screening procedures.
2. End of Study (EOS) is at the Week 52 visit. If a participant discontinues the study prior to week 52, EOS procedures must be performed within the timeframe of the next scheduled visit. If EOS does not occur at Week 52, see footnote 23.
3. Any follow-up visit requiring a physical exam must be performed at the study center. All other visits without a physical exam may be performed by a qualified and trained in-home service provider.
4. Informed consent must be obtained prior to any study-related procedures.
5. Screening genotype and/or human leukocyte antigen (HLA) samples collected only if not known. Investigator and participant to review age-appropriate vaccinations during the screening period.
6. Assessments are completed between Screening visit and prior to Dose Day 0. Lab collection for vector shedding is allowed on Day 0 pre-SPK-8016 dosing.
7. Height measured only at Screening.
8. At Day 0, weight obtained from Screening (or the weight obtained from the most recent visit prior to infusion) will be used to calculate the dose of FVIII product and SPK-8016. Physical Exam is comprehensive (not targeted), at all designated visits.
9. At Days 0 and 1, vital signs (i.e., blood pressure, pulse, respiratory rate, and oral/temporal temperature), are measured after the participant has been resting upright or supine for approximately 5 mins at the following timepoints: SPK-8016: Pre-infusion and post-infusion of SPK-8016 (i.e., ±2 min, 2 hrs (±10 mins), and 24 (±1) hr).
10. Screening serology will be performed as follows: For all participants: HBsAg, anti-HBc, HBV-DNA; HCV-RNA load assay, HIV-1/HIV-2 Ag/Ab; For HIV-positive participants: CD4+ count and HIV-1/HIV-2 viral load. LL results may be used for eligibility assessment.
11. Screening liver function tests (LFTs) and CRP performed by CL and LL. LL results may be used for eligibility assessment. LFTs on 3-7 days Prior to Day 0 are performed by the local lab only. Twice weekly monitoring of AST, ALT, ALP, GGT, LDH, FVIII:C, and FVIII Ag will be collected during Days 3 through 77 post vector-infusion visits.
12. Screening ECG required for participants > 50 years of age, or if clinically indicated.

13. Screening Fibroscan (LL), FibroTest/Fibrosure (CL), or AST-to-Platelet Ratio Index (LL) is required for participants without known pre-existing diagnosis of portal hypertension, splenomegaly, or hepatic encephalopathy. For Liver Ultrasound, "if indicated" means "if, in the judgment of the investigating site or the Sponsor, the liver ultrasound is indicated to aid interpretation of the screening evaluation of liver fibrosis".
14. Lipid panels (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides) for participants with a history of dyslipidemia or hypercholesterolemia, or if clinically indicated.
15. Vector shedding: polymerase chain reaction (PCR) analysis on serum, saliva, urine, and semen will be collected at Screening or Day 0 (prior to vector infusion) and weekly starting at Day 3 post vector infusion and continuing until 3 consecutive samples are negative (classified as: at or below the limit of detection of the assay).
16. The morning of Day 0, or prior to [redacted] for cohort 2, participants will administer a single prophylactic IV infusion of approximately 50 IU/kg of FVIII product. This may be self-administered and recorded in the subject infusion log. Site assistance is permitted for the FVIII infusion
17. Day 0 blood samples for FVIII activity, FVIII antigen, and aPTT will be collected pre-SPK-8016 infusion.
18. Participants will receive a single IV infusion of SPK-8016 for approximately 60 minutes.
19. Quality-of-life questionnaires (Hemophilia Activities List, Haem-A-QoL, EQ-5D-5L) will be completed by the participants.
20. Training and dispensation of FVIII Infusion Log at Day 0, collect, review, and dispense at subsequent visits.
21. Spare plasma will be collected for further coagulation assays, future research, or for clarification of any clinical or laboratory AEs.
22. Unscheduled visits or safety tests may be performed for safety monitoring purposes or repeat safety assessments.
23. If the long-term follow-up (LTFU) study is not enrolling at the time of a participant's planned Week 52 visit, the participant may remain in SPK-8016-101. The procedures from Week 46, except for collection of immunology samples for ELISpot, will be performed at Week 52 every 12 weeks until the LTFU study is open. At that time, the week 52/EOS visit should occur. The Week 52 procedures are to be performed only at the EOS Visit.
24. Any concomitant medication that has known hepatoxicity should be discontinued in the first 12 weeks following vector infusion.
25. Local hematology, local liver function, and central immunology and immune profiling must be obtained 3 to 7 days prior to Day 0. A remote service provider may be used for this visit. Contact the participant to verify no changes in the general health status prior to first dose of [redacted]
26. Hematology should be monitored weekly and lipid profile monthly for Cohort 1 and 2 until [redacted] dosing has stopped. The frequency may be altered based on clinical response.
27. In Cohort 2, [redacted] following Day 0 and may be considered based on clinical/immune response in consultation with the Sponsor. See Section 4.1.1
28. In Cohort 1, [redacted]
29. Participants in cohort 1 and 2 will be retested at Day 84 for HBsAg and anti-HBc.
30. Immune profiling will be collected prior to the immunomodulation [redacted] 3-7 days prior to Day 0, pre-administration of SPK-8016, and 30 (± 2) minutes, 2 hr (± 10 min), 5 hr (±10 min), and 24 (± 1) hours post vector infusion, and Days 7, 14, 21, 28, 56, and Week 16.
31. PAX gene will be collected prior to the immunomodulation [redacted], pre- administration of SPK-8016, 5 hr (± 10 min) and 24 (± 1) hours post vector infusion, and Days 14, 28, and 56.
32. If an apparent CTL immune response/liver inflammation is observed and triggers the initiation of reactive corticosteroids, please collect additional samples for "Immune Profiling" and "PAX gene" at the following times: prior to initiating [redacted] corticosteroids or oral corticosteroids (if possible, without delaying the initiation of corticosteroid therapy) and then week 1 (± 2 days) and Week 2 (± 2 days) following initiation of corticosteroids.
33. Anti-SARS-CoV2 serology testing at baseline and at week 18. At the discretion of the Investigator, Nucleic acid amplification testing (NAAT) for infection with SARS-CoV2 may be performed 3 to 7 days prior to Day 0 (Cohort 2 only) and prior to the initiation of reactive corticosteroids.
34. If the PK Profile is not available in historical records on the current FVIII product, the participant must undergo a PK analysis locally at Screening or Day 0.
35. Fibrinogen antigen, Thrombin Time (TT), D-dimer are required at Screening, Day 10 and Day 24 for participants enrolled in Cohort 2.

2 INTRODUCTION

2.1 Background

SPK-8016 is an investigational gene therapy medicinal product that contains [redacted]

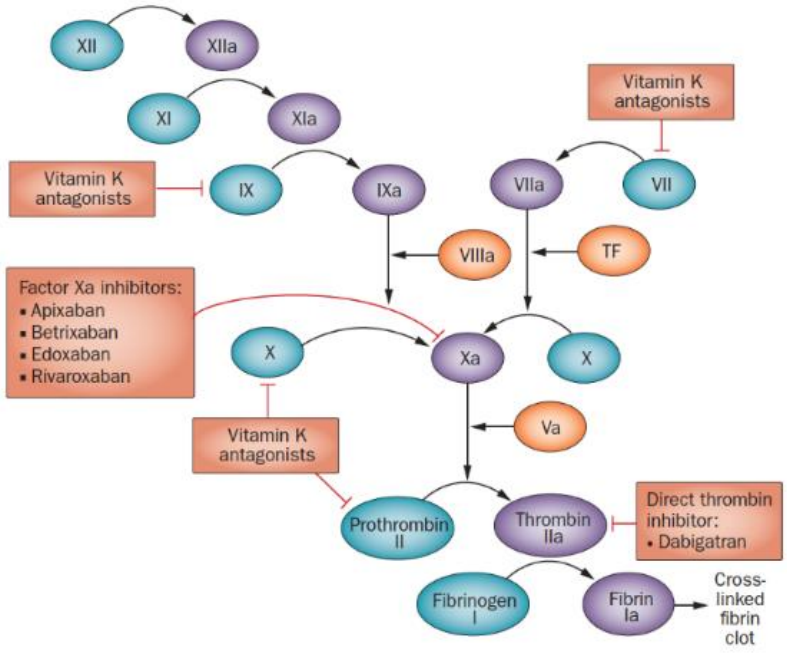
SPK-8016 is being developed by Spark Therapeutics for the treatment of hemophilia A.

This clinical study (SPK-8016-101) will evaluate the safety, efficacy, and tolerability of SPK-8016 in adult males with clinically severe hemophilia A and no measurable inhibitor against FVIII. Data obtained from this study will inform the design and dose selection for subsequent studies of SPK-8016 in other patient populations (e.g., those with inhibitors).

2.1.1 Hemophilia A

Hemophilia A is an X-chromosome linked bleeding disorder that primarily affects 1 in 5,000 male births (Giangrande, 2005; Srivastava, 2013). The severity of disease is characterized by the endogenous level of FVIII measured in the plasma. Severe hemophilia A is defined as a coagulation activity of FVIII in plasma (FVIII:C) of <1% (<1 IU/dL) of normal levels. Factor VIII, a single chain glycoprotein pro-cofactor, is composed of 6 putative domains arranged in the order of a heavy chain (A1, A2, and B regions) and a light chain (A3, C1, and C2 regions). The light chain (Lollar, 1988) contains binding sites for von Willebrand factor (VWF), activated protein C (Walker, 1990), activated factor IX (FIXa), and phospholipid (Foster, 1990). FVIII and VWF circulate in plasma as a non-covalently-linked complex that stabilizes the intrinsically unstable FVIII protein. Observations that the FVIII-VWF complex is dissociated by phospholipids and that VWF prevents FVIII from binding to phospholipids and platelets suggest antagonism between phospholipids and VWF for binding to FVIII. Although VWF and FVIII are glycoproteins, they have different roles in the initiation and regulation of hemostasis. VWF is necessary for mediating platelet-vessel interactions at the site of vascular injury. Thrombin and FIXa each activate FVIII functions by cleaving the light chain at amino acid residue 1689. This cleavage releases FVIII from VWF and allows binding of FVIII to phospholipid resulting in the formation of the tenase-activated factor X (FXa) complex. This is the central step of the coagulation cascade. The coagulation cascade has 2 pathways: the Activation Pathway (Intrinsic Pathway) and the Tissue Factor Pathway (Extrinsic Pathway) (Makaryus, 2013). The plasma factors normally circulate in inactive forms and are activated in a cascade or "waterfall" of amplifying reactions (Macfarlane, 1964; Davie, 1964) one after the other until the soluble plasma protein, fibrinogen, is transformed into a fibrinous clot. The blood coagulation cascade is illustrated in Figure 2.

Figure 2: The Blood Coagulation Cascade



Although platelets are critical to the formation of the hemostatic plug, an effective clot cannot be formed without adequate levels of pro-coagulant factors. In normal individuals, the level of coagulation factors in the plasma ranges from 50 - 150% (or 50 -150 IU/dL) of the level in normal pooled plasma. Therefore, in hemophilia, clinical features and factor coagulant activity define the severity of the disease.

2.1.2 Clinical Manifestations

About 70% of newborn babies with hemophilia have a positive family history. When the diagnosis is not suspected based on a positive family history, affected children present with bleeding from the umbilical stump, prolonged bleeding after circumcision, bleeding following intramuscular immunization, excessive bruising, or rarely with intracranial hemorrhage. Individuals with FVIII:C $\leq$ 2% of normal experience frequent life-threatening spontaneous and traumatic bleeding, particularly in joints, soft tissues, and muscles. When inadequately managed, musculoskeletal hemorrhages can lead to recurrent hemarthroses (chronic arthropathy) and the development of target joints (the generally accepted criterion is a minimum of 3 bleeds into a single joint within a consecutive 6-month period (Blanchette, 2014). The inevitable result of such bleeding events is progressive joint damage, leading to disabling arthritis with major effects on physical and psychosocial quality-of-life (QoL) and socio-economic parameters for hemophilia patients (Fogarty, 2011). Intracranial hemorrhage is a leading cause of death among individuals with hemophilia, with a mortality rate of up to 50% in adults, as well as in children. An intracranial hemorrhage can occur after trauma, but as many as 50% of cases occur spontaneously (Stieltjes, 2005).

Development of alloantibodies to FVIII (i.e., inhibitors) is the main complication of any factor replacement therapy (Goudemand, 2006; Kessler, 1991; White, 2005). Inhibitory antibodies to

FVIII (usually immunoglobulin G subclass 4 [IgG4] antibodies that neutralize the procoagulant activity of FVIII or coagulation factor IX [FIX]) are estimated to occur in 20 to 35% of individuals with severe and mild-to-moderate hemophilia A, respectively (Gouw, 2013; van den Berg, 2013; Mancuso, 2012; Hay, 2011), following exposure to factor replacement therapy. In the presence of these inhibitory antibodies, replacement of the missing clotting factor by infusion of FVIII becomes less effective. Once replacement therapy becomes ineffective, the acute management of bleeding requires agents that bypass FVIII activity. Long-term management of inhibitors in hemophilia A typically consists of eradicating the inhibitor through immune tolerance. Therefore, development of inhibitors significantly adds to patients' disease burden.

2.1.3 Current Therapies and Prevention for Hemophilia A

2.1.3.1 Current Therapies

There is no available cure for hemophilia A. Factor replacement therapy, purified from human plasma, first became available more than 4 decades ago. Although these FVIII products dramatically improved life expectancy and QoL in the U.S.A. and Western Europe, they also resulted in exposure of individuals with hemophilia to blood borne viruses – most significantly hepatitis B, hepatitis C, and the human immunodeficiency virus (HIV). HIV sero-conversion studies documented that most individuals with hemophilia were infected between 1978 and 1984 (Eyster, 1985; Ragni, 1986; Ragni, 1987). In the U.S.A., most patients with severe hemophilia who were born before 1987 are HIV positive, and many have already died from complications related to the acquired immune deficiency syndrome (AIDS). The majority of persons with hemophilia A born before 1987 are affected by hepatitis C virus (HCV) disease which can cause chronic and progressive hepatitis, with eventual development of cirrhosis. Late complications of HCV are an increasing cause of death in adults who have been infected for decades (Brettler, 1990; Darby, 1997).

In the 1990's, concerns about viral contamination (Mannucci, 2001) prompted the development of high-purity virus-inactivated plasma-derived products and genetically engineered recombinant factors with no animal- or human-plasma-derived proteins to minimize the risk of disease transmission (Roth, 2001; White, 1998). Indeed, since the introduction of effective virus inactivation procedures, there have been no documented transmissions of hepatitis B and C viruses, HIV, West Nile, or malaria (Tabor, 1999), although no pathogen inactivation process has been shown to eliminate all pathogens (such as rare reports of infectious prions and parvovirus transmission). However, these products have not circumvented all the problems of protein-based therapies (Mannucci, 1993a; Mannucci, 1993b).

Current treatment of the disease is based on venipuncture and intravenous administration of either plasma-derived or recombinant FVIII (rFVIII) protein replacement home therapy to raise the circulating FVIII (FVIII:C) activity level to the lowest effective dose to achieve either resolution of bleeding (on-demand treatment) or prevention of bleeding (prophylaxis treatment) (Roberts, 1993; Srivastava, 2013; National Hemophilia Foundation, 2007). Venous access via peripheral veins remains the preferred option for the administration of FVIII products (Komvilaisak, 2006) because it allows a large amount of product to be administered frequently as a short infusion using small needles (23-25 gauge). The frequency of administration of FVIII products varies among individuals and is tailored to the individual's clinical status, taking into

consideration the type of bleeding, frequency of bleeding, and goal of treatment for the participant. Both the U.S. National Hemophilia Foundation and the World Federation of Hemophilia (Srivastava, 2013) established recommendations of plasma factor levels and duration of administration for different types of bleeds based on observations over the years. Improvements in FVIII replacement therapy have vastly increased the QoL and life expectancy of individuals with hemophilia A; and a recently licensed modified FVIII agent with an extended half-life (EHL) (Mahlangu, 2014) provided more convenient dosing options (Lambert, 2007).

2.1.3.2 Current Prevention

Chronic arthropathy is the major morbidity of hemophilia resulting from recurrent spontaneous bleeds into the joints. Many studies have shown that, even at high doses, on-demand therapy is not effective in preventing arthropathy (Petrini, 1991; Aledort, 1994). Thus, scheduled protein replacement to prevent bleeding (prophylactic treatment) as the standard of care for hemophilia rather than on-demand treatment was facilitated by the introduction of rFVIII concentrates in the early 1990's. Prophylactic treatment (regular IV infusions ranging from twice a week up to every other day) aims to maintain plasma FVIII levels ≥ 1%, thereby changing the expected phenotype from severe to moderate hemophilia (Nilsson, 1992). Observations of individuals with moderate hemophilia, and a generation of clinical research in hemophilia patients treated prophylactically with clotting factor replacement, have documented that minimal elevations in the levels of normal circulating clotting factor activity ≥1% are sufficient to prevent bleeding, and this has been demonstrated in the Swedish prophylactic studies (summarized in Löfqvist, 1997; Ljung, 1998; and Nilsson, 1992).

Prophylactic therapy, which aims to convert the severe phenotype to moderate through regular infusions of clotting factor, with 100% adherence to dosing regimen, is more effective than on-demand treatment at preserving joint health (Manco-Johnson 2007; Gringeri, 2011). It has revolutionized health outcomes in hemophilia by enabling affected individuals to participate in physical activities (Wang, 2016; Negrier, 2013), and natural history data suggests that FVIII:C around 12% of normal levels may be sufficient to protect from spontaneous joint bleeds (Mahdi, 2015; den Uijl, 2011). However, regular replacement therapy poses significant challenges for the hemophilia community, including the frequency of IV infusions required, necessity to adhere rigorously to the prophylactic regimen, variability in individual pharmacokinetics (PK) requiring personalized regimens, potential development of neutralizing alloantibodies ("inhibitors"), and cost-effectiveness.

Prophylactic therapy for hemophilia has gradually increased among the adult population in the U.S.A. As of 2014, an estimated 85% of children and 63% of adults with hemophilia were on prophylactic regimens (World Federation of Hemophilia (WFH) Annual Global Survey 2014, 2015). However, it is still not universally practiced for several reasons. The expense of prophylaxis may be prohibitive; the cost of prophylactic treatment for an adult (70 kg) can be as high as $630,630 annually using the currently available EHL FVIII fusion proteins (Croteau, 2015). Furthermore, the burden of treatment on patients is great. Prophylaxis with either regular or EHL rFVIII protein (half-lives range from 10.8 -19.7 hours) typically requires 2-3.5 infusions per week (National Hemophilia Foundation - Medical and Scientific Advisory Council [NHF-MASAC] Recommendations Document #240). In a 2001 survey of 34 hemophilia patients on prophylaxis, only 60% reported infusing at least three fourths of the recommended factor,

commonly missing doses due to time commitment and complexity (Hacker, 2001). Recent results from a multi-center study assessing adherence to prophylaxis and clinical outcomes in the Netherlands revealed only 43% of the patients adhered to the prophylactic regimen (Schrijvers, 2016), despite the fact that poor adherence resulted in significantly greater numbers of bleeding episodes and lower physical health status (Krishnan, 2015).

2.1.4 Alternative Therapy for Hemophilia A

In the case of hemophilia, where a cure is currently not attainable and lifelong therapy is needed, QoL is an essential outcome parameter. All hemophilia prophylaxis studies using health-related quality-of-life (HR-QoL) score as an outcome reported a decreased HR-QoL compared with the general population and an improvement with prophylactic treatment (Royal, 2002; Fischer, 2003a; Fischer, 2003b). Although the World Health Organization (WHO) has advised the continuation of prophylactic treatment for life, its establishment has economic and practical hurdles. The goals of an alternative approach are to reduce short-term disability and long-term joint damage and to improve patients' overall QoL and functional independence (Colvin, 2008).

2.1.4.1 Factor VIII and Protein

The human FVIII gene is located on the long arm of the X chromosome, at Xq28 (Poustka, 1991; Freije, 1992). It spans 186 kb and consists of 26 exons separated by 25 introns (Gitschier, 1984; Toole, 1984). Most of the exons that make up the 9 kb mRNA are small (69-262 bp), with the exception of exon 14 (3.1 kb) and exon 26 (1.9 kb), which primarily consist of the 3' untranslated sequence. The resulting 7 kb coding sequence encodes a 2351 residue single chain precursor protein. Following cleavage of a 19 amino acid signal peptide, the mature 2332 amino acid protein is produced, which has a domain structure of A1-A2-B-A3-C1-C2. The 3 homologous A domains bind Ca+2 and are essential for FVIII catalytic cofactor activity (Tagliavacca, 1997). There are short acidic sequences (a1, a2, a3) between A1 and A2 and at the A2-B and B-A3 junctions, respectively. These regions are close to important proteolytic cleavage sites, contain tyrosine residues that are sulfated, and may affect the interaction of FVIII with other components of the coagulation pathway (Mumford, 2002; Pittman, 1992). The large central B domain is encoded by exon 14, is heavily glycosylated and is not necessary for activity (Pittman, 1993; Eaton, 1986). The acidic region (a3) that follows the B domain contains a major VWF binding site (Foster, 1988), and the 2 C domains are responsible for FVIII binding to phospholipids (Arai, 1989).

Single chain FVIII is proteolytically processed to generate a heavy chain that is composed of domains A1-A2-B and a light chain that is composed of domains A3-C1-C2. These chains circulate in an inactive state bound to VWF as a heterodimer. Activation of FVIII occurs following thrombin cleavages between domains A1-A2 and A2-B, resulting in release of the B domain and formation of a heterotrimer containing the A1, A2 and A3-C1-C2 domains.

The full length FVIII protein is encoded by a 7 kb DNA sequence, which exceeds the packaging limit of an AAV vector (~4.7 kb). However, since the [redacted] is not required for FVIII activity (Toole, 1986), the 3.1 kb DNA sequence encoding the [redacted] can be eliminated, resulting in an ~4 kb coding sequence that produces a [redacted] but active FVIII protein. [redacted]

[redacted]

The natural site for the biosynthesis of FVIII has been the subject of debate for decades. Although it has been clear that liver cells contribute significantly to circulating FVIII levels, since liver transplantation corrects FVIII levels in hemophilia patients (Bontempo, 1987), the specific cell type responsible for biosynthesis and secretion of the protein has been controversial. Early after isolation of the gene, it was thought that FVIII mRNA and protein were co-localized in hepatocytes (Wion, 1985; Zelechowska, 1985). However, based on improved cell separation and sorting techniques, it is now clear that liver sinusoidal endothelial cells (LSECs) are the natural site of FVIII biosynthesis (Shahani, 2014). At the present time, no rAAV vectors that efficiently target LSECs are available. Therefore, [redacted]

2.1.4.2 Biology of Adeno-Associated Virus (AAV) Vectors

AAV is a non-enveloped, replication-defective parvovirus that has not been associated with human disease. AAV vectors are derived from the parent virus by removing all of the viral elements except for the inverted terminal repeats (ITR) and inserting the gene or genes of interest and their associated regulatory elements (Samulski, 1982; Samulski, 1987). The long-term safety of these vectors in humans is unknown; however, AAV vectors have been delivered to hundreds of human participants in trials for cystic fibrosis, rheumatoid arthritis, inherited retinal degeneration due to autosomal-recessive retinal pigment epithelium 65 (RPE65) gene mutations, α_1-antitrypsin deficiency, as well as hemophilia, and have been remarkably free of vector-related adverse events (Mingozzi, 2011a; Mingozzi, 2013a). Thus, AAV vectors are one of the most efficient in vivo gene delivery platforms. In October 2012, the European Commission granted marketing authorization for Glybera® under exceptional circumstances as a treatment for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) confirmed by genetic testing and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions. This was the first AAV-based vector product granted marketing authorization in an International Conference on Harmonisation (ICH) region.

AAV vectors do not require actively dividing target cells to achieve efficient transduction, as demonstrated in post-mitotic cells of brain, muscle, liver, and retina in vivo (Maguire, 2008; Maguire, 2009; Mingozzi, 2011b; Mingozzi, 2013b; Mingozzi, 2011a). Also, at least in animal studies, there is no immune response directed against the transduced cell, likely because all the viral genes have been removed. This absence of immune response accounts, at least in part, for prolonged (months to years) transgene expression observed in animals following a single administration of an AAV vector. Several groups have established that AAV efficiently transduces hepatocytes following a single administration via the portal vein, hepatic artery, or IV route, resulting in long-term (more than 10 years), dose-dependent transgene expression in large animals including dogs and NHPs (Snyder, 1997; Nakai, 1998; Xiao, 1998; Jiang, 2006;

Niemeyer, 2009; Nathwani, 2011a). A memory CD8+ T cell response to AAV in humans would be expected to respond more efficiently to partially degraded AAV capsid peptides displayed on the cell surface of transduced hepatocytes in the context of major histocompatibility complex (MHC) Class I molecules (Manno, 2006). Indeed, studies document expansion of a population of AAV-capsid specific CD8+ T cells after AAV vector infusion into the hepatic artery in humans, whereas no such expansion is seen in animals receiving an AAV vector (Mingozzi, 2007). Thus, humans' previous exposure to wild-type AAV likely accounts for the differences between humans and all other species in immune response to capsid. Although NHPs also have prior exposure to wild-type AAV, they fail to mount recall T cell responses to AAV capsid antigens in spite of readily detectable AAV capsid-specific T cells at baseline. Ertl and colleagues have shown that AAV capsid-specific T cells from rhesus macaques show marked differences in function and differentiation status compared to those found in healthy human adults (Li, 2011), which may explain their differential reactivation by AAV vectors.

2.1.4.3 Gene Therapy as an Alternative Approach

As a proposed alternative approach, gene therapy may potentially reduce short-term disability and long-term hemophilic arthropathy, reduce incidence of central nervous system (CNS) bleeding, eliminate the need for indwelling intravenous catheters or frequent factor infusion, and improve participants' overall QoL and functional independence (Colvin, 2008). Preliminary results of a hemophilia B study, sponsored by St. Jude Children's Research Institute-University College London, demonstrate a reduction in the prophylactic use of factor replacement therapies in 10 participants who received gene transfer. Additionally, at least 5 of these participants have reduced factor consumption by greater than 90% while remaining free of spontaneous bleeding episodes (Nathwani, 2011b; Nathwani, 2014).

Several features make hemophilia A good model for gene therapy. First, the precise regulation of transgene expression is not required. The therapeutic range is remarkably wide, from >1% to 150% of normal. Data from administration of FVIII products into patients with hemophilia A demonstrate clearly that levels ≤150% are not associated with ill-effects since the protein circulates as a zymogen (inactive precursor). Second, as stated, >1% circulating FVIII levels may provide protection against chronic arthropathy and CNS bleeding. Patients with levels >5% have mild severity and only rarely experience spontaneous bleeding episodes (although they exhibit abnormal bleeding in response to hemostatic challenges such as surgery or trauma). Third, hemophilia A has available both large and small animal models of the human disease, a major asset in efforts to establish an experimental basis for gene therapy. There are well characterized, naturally occurring canine models of the disease and genetically engineered hemophilic mice (Evans, 1989a; Evans, 1989b; Connelly, 1996; Bi, 1995; Lin, 1997). Fourth, determination of therapeutic efficacy is straightforward and unequivocal in the case of hemophilia A since plasma levels of FVIII are easy to measure and correlate well with clinical disease severity.

2.2 Rationale

The primary aim of hemophilia care is to prevent and treat bleeding due to deficient clotting factors. Both hemophilia A and B can be treated with recombinant factor replacement leading to significant improvement in morbidity and mortality. However, such treatment is extremely costly

and is often still associated with clinical complications that include bleeding, particularly bleeding into the joints (hemarthrosis). Moreover, this treatment requires recombinant factor infusions 2 to 3 times per week to maintain minimal therapeutic levels; thus, it is demanding and invasive.

Since genes were initially cloned more than 30 years ago, gene therapy has been the goal for curative treatment of hemophilia. As a novel alternative to the current treatment for hemophilia A, gene therapy could potentially reduce short-term disability and long-term hemophilic arthropathy, reduce incidence of CNS bleeding, eliminate the need for indwelling intravenous catheters or frequent factor infusion, and improve patients' overall QoL and functional independence (Colvin, 2008). Indeed, recent studies have shown that liver mediated gene therapy with AAV vectors can safely and consistently achieve sustained clinically meaningful levels of FVIII activity and can potentially eliminate spontaneous hemarthrosis.

SPK-8016 (adeno-associated viral vector with a [redacted] is being developed by Spark Therapeutics for the treatment of hemophilia A. This clinical study (SPK-8016-101) will evaluate the safety, efficacy, and tolerability of SPK-8016 in adult males with clinically severe hemophilia A and no measurable inhibitor against FVIII. The data obtained from this study will inform the design and dose selection for subsequent studies of SPK-8016 in other patient populations (e.g., those with inhibitors).

In the Western world, the hemophilia population with the greatest unmet need are patients who develop inhibitors, or neutralizing antibodies, to FVIII. For these patients, a cornerstone of treatment is the eradication of these neutralizing antibodies after they develop, and data in animal models of hemophilia support the possibility of using gene therapy to carry out this eradication.

2.2.1 Description of SPK-8016

SPK-8016 is a rAAV vector composed of [redacted] and [redacted]. The full length FVIII protein is encoded by a 7 kb cDNA sequence, which exceeds the packaging limit of an AAV vector (~4.7kb). Thus, the Sponsor has chosen [redacted]

A number of measures have been taken to improve the expression and safety of the transgene product compared with the previous vector iterations tested by the Sponsor or by others (Figure 3).

a) Capsid:

- [redacted]

b) Cassette:

- [redacted]
- [redacted]

Figure 3: Schematic of SPK-8016 vector genome

[redacted]

It has been previously demonstrated that excess empty capsids may adsorb low-level neutralizing antibodies and non-neutralizing antibodies, permitting liver transduction after peripheral vector infusion even in their presence (Mingozzi, 2013b). This may be particularly important at lower vector doses. This hypothesis is consistent with clinical data from previous hemophilia B gene therapy trials in which the presence of empty capsids in the formulation correlated with higher expression levels at low vector doses (Monahan, 2015; Nathwani, 2014). For this reason, [redacted], comparable to the Sponsor's recent clinical trial (Investigational New Drug [IND] [redacted]) for hemophilia B (High, 2016), as well as the Sponsor's ongoing SPK-8011-101 clinical study in patients with severe hemophilia A.

SPK-8016 is manufactured according to good manufacturing practices (GMP) guidelines and will be administered to male individuals with severe hemophilia A (Section 5.1).

2.2.2 Summary of Non-Clinical Experience with SPK-8016 or Other Relevant Spark AAV Vector

[redacted], another vector developed by the Sponsor and currently under clinical investigation in a Phase 1/2 study for the treatment of hemophilia A (NCT03003533, [redacted]). [redacted]

[redacted] Given the similarities between the 2 vectors, non-clinical studies with SPK-8011 are relevant to support the safety and potential efficacy of SPK-8016 in humans and are described below.

As discussed in the Investigator's Brochure (Section 3.1), the non-clinical testing for SPK-8016, as well as other Spark gene therapy vectors for hemophilia A (e.g., SPK-8011), has been complicated by 2 major observations:

- [redacted] transduces mouse hepatocytes poorly, yielding extremely low levels of liver-directed transgene expression following intravenous administration. Consequently, mouse models are inappropriate to evaluate the safety of hepatic transgene expression derived from vectors of this AAV serotype.
- [redacted]

Based on these findings, it was necessary to adopt a nonconventional testing strategy that involved evaluating the safety of sustained expression of the transgene (hFVIII) products using [redacted] in 1 species (mice) and the safety of the novel [redacted] in another species, NHPs, as it is not possible to evaluate the safety of both sustained hFVIII expression and capsid with a single vector in a single species.

Specifically, for SPK-8016, 2 non-GLP safety and pharmacology studies were performed in cynomolgus macaques to evaluate the safety of the vector capsid and compare short-term potency of different Spark AAV vectors.

[redacted]

[redacted]

Preliminary information on the biodistribution of the [redacted] capsid in NHPs demonstrates that the vector disseminates mostly to the liver and spleen, with more than 1000-fold less vector observed in other tissues. A separate study designed to evaluate the potential for germline transmission of the [redacted] was performed in rabbits using a vector encoding the hFIX transgene. The [redacted] showed very limited distribution to semen, even at doses as high as $1x10^{13}$ vg/kg. This pattern differs from other vectors investigated including AAV2, AAV8, and [redacted] potentially making this rAAV capsid safer, in terms of genotoxicity, than the others.

2.2.3 Summary of Clinical Experience with SPK-8016 or Other Relevant Spark AAV Vector

This study (SPK-8016-101) is the first clinical study to administer SPK-8016 to humans. However, [redacted], has been administered to 17 patients with hemophilia A in an ongoing clinical study (SPK-8011-101; a summary of preliminary results is provided in Section 2.2.3.1). Additionally, gene therapy with rAAV vectors has been administered to humans in more than 100 gene transfer clinical trials for a wide range of indications, including multiple studies in hemophilia A and B (George, 2017; Rangarajan, 2017; Miesbach, 2017; Pasi, 2020; Nathwani, 2014; Nathwani, 2011b; Manno, 2006; Monahan, 2015; Mingozzi, 2011a; Mingozzi, 2011b).

[redacted]

[redacted] One participant did not require immunosuppressive therapy. No participant developed FVIII inhibitors. No serious adverse events (SAEs) or increase in liver transaminases above the upper limit of normal (ULN) related to the vector were observed.

2.2.3.1 Summary of Overall Clinical Experience with AAV-Mediated Gene Therapy

Based on early experience with SPK-8016, SPK-8011, as well as other AAV-mediated gene transfer trials for hemophilia A and B, the following preliminary observations can be made:

- AAV-based strategies for hemophilia have resulted in sustained expression of clotting factor at levels sufficient to reduce or eliminate the need for clotting factor infusions after a single IV infusion of the AAV-mediated gene.
- Neutralizing antibodies against FVIII or FIX have not been reported for any participant to date.
- [redacted]
- [redacted]
- [redacted]

Although the early results are promising, additional investigation is needed to determine whether there is a transient immunosuppressive regimen that will reliably control immune responses in all patients and allow long-term expression of the donated gene. Based on prior clinical studies, non-clinical studies summarized here, and input from clinical experts in rheumatology, organ transplantation, viral immunology, and bone marrow transplantation, the 2 prophylactically administered immunomodulatory regimens shown in Figure 1 will be evaluated. [redacted]

2.2.4 Summary of Non-clinical and Clinical Experience with [redacted]

In order to test whether [redacted] a non-good laboratory practice (GLP) study in NHPs (Study# IMM_PC2019_001/Covance 8400655) was performed. One of the aims of the study was to rule out the possibility of [redacted] blockade increasing the percent of animals developing anti-transgene antibodies, as observed with other immunosuppressive drugs (Mingozzi, 2007). However, since virtually all NHPs will develop neutralizing antibodies against human FVIII following gene transfer (McIntosh, 2013), this assessment would be precluded if a FVIII cassette were used. To this end, FIX was selected as the transgene of choice, as only approximately one third of NHPs develop antibodies against AAV-derived human FIX. An AAV vector serotype, [redacted] was used to express the human coagulation factor IX (hFIX) transgene in the livers of healthy male cynomolgus macaques under the control of a liver-specific promoter. Ten Spark100-seronegative animals were divided into 2 experimental groups (5 per group). One of the groups received [redacted] 1 day prior to vector infusion. [redacted]

No animals demonstrated hypersensitivity or anaphylactic reactions following administration of either [redacted] or [redacted]. Animals were bled every week for analysis of safety endpoints and hFIX transgene expression levels, along with monitoring [redacted] The study had a follow up duration of 13 weeks post vector infusion. All animals survived to their scheduled sacrifice. The development of subacute decreases in neutrophil counts following single dose [redacted] has been observed in human healthy volunteers; no animals in this study developed neutropenia or thrombocytopenia below baseline. Two of 5 animals developed isolated transient elevations of liver transaminases (<2 x upper limit of normal (ULN)) which resolved without intervention without evidence of hepatic obstructive disease

(hyperbilirubinemia) or hepatic insufficiency. Importantly, clinical and anatomic pathology examination of the liver and a panel of 40 organs and tissues showed no macroscopic or microscopic findings in animals receiving the [redacted] vector alone or in combination with [redacted]. Hepatic transduction as assessed by vector genome copy number in liver (at sacrifice) and by circulating hFIX antigen levels (weekly throughout the study) was not significantly different in the animals that received [redacted] compared to controls.

[redacted], and in general innate immunity driven by the AAV capsid (Hösel, 2012; Kuranda, 2018) and DNA payload (Martino, 2011; Rogers, 2017), are key triggers of AAV vector immunogenicity including adaptive humoral and cellular responses. Several observations from this study suggest that animals that received [redacted]

With regard to cellular immune responses, prior studies performed using human CD8+ and CD4+ T lymphocytes identify TNFα as one of the main cytokine signatures of T cell activation in response to AAV (Kuranda, 2018). Thus, peripheral blood mononuclear cells (PBMC) from control animals and [redacted]

2.2.5 Summary of Non-Clinical and Clinical Experience with [redacted]

Pre-clinical studies of hepatic artery administration of AAV8-hAAT-hFIX16 were performed in rhesus macaques; in these studies, vector was given alone or together with a short course of

immunomodulation to assess the potential safety and efficacy of the approach. [redacted]
The [redacted] and contained the wild type copy of the hFIX cDNA.

[redacted]

The regimen of [redacted] The side effect profile was acceptable and there were no SAEs on this regimen. The side effects of immunomodulatory drugs are well described (Post, 2005; Perry, 2005) and include the risk of hepatitis virus reactivation (Savas, 2007; Melon, 2005; Lalazar, 2007; Francisci, 2006; Calabrese, 2006) and the occurrence of B cell lymphoproliferative disorders (Gross, 1999; Pascual, 2007; Bakker, 2007). [redacted]

[redacted]

2.3 Benefit/Risk Assessment

2.3.1 Risk Assessment

The following are potential risks with the administration of any rAAV gene therapy for hemophilia and they will be monitored in this study. Participants should be advised to notify the Investigator(s) immediately and/or seek immediate emergency care, depending on the severity of the reaction, if any symptoms occur.

2.3.1.1 Allergic Reaction or Anaphylaxis

There has been 1 report of symptoms associated with SPK-8011 (rAAV-FVIII) infusion, occurring following SPK-8011 vector infusion at a dose of $2x10^{12}$ vg/kg. Nonserious events including vomiting, pyrexia, back pain, and myalgia were reported in 1 participant; events started to resolve within 12 hours (per Investigator), all completely resolved in 1 to 3 days with acetaminophen (High, 2018). Participants should be informed of early symptoms and signs of

hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. If such an event occurs, the participant should be instructed to seek immediate medical care.

2.3.1.2 Inhibitor Development

There is a possibility that participants infused with SPK-8016 will develop inhibitors to FVIII, although inhibitor development has not occurred in any participants who have received AAV2-hFIX, AAV8-hFIX (Nathwani, 2014), AAV8-hFIX19 [redacted] or BAX-335 (under National Clinical Trial (NCT) 01687608). In addition, inhibitor development has not been reported in any of the 17 participants in the Sponsor initiated Hemophilia A gene therapy study SPK-8011-101. Moreover, no inhibitor development has been reported in the recent BioMarin Hemophilia A gene therapy trial of an AAV5-BDD-hFVIII (BMN-270) (Rangarajan, 2017; Pasi, 2020). Inhibitor development most often occurs within the first 20 infusions of FVIII replacement therapies. The likelihood of participants in this study developing inhibitors will be minimized by including only individuals who have not made inhibitor antibody to FVIII, despite prior heavy exposure to the FVIII infused protein (i.e., those who have had greater than 150 exposure days to FVIII concentrates). Blood samples will be taken at regular intervals to monitor for inhibitor formation during the study and archives will be maintained for possible future analysis should inhibitor development occur.

2.3.1.3 Elevation of Hepatic Transaminases

None of the 4 enrolled participants in study SPK-8016-101 experienced adverse events of transaminitis related to SPK-8016. Of the 17 participants enrolled in the Sponsor's similar hemophilia A gene therapy trial SPK-8011-101, 5 (29%) reported SPK-8011-related adverse events of increase of ALT/aspartate aminotransferase (AST) with severity ranging from mild to moderate in the higher dose groups (2 in the $1.5x10^{12}$ vg/kg dose cohort, and 3 in the $2x10^{12}$ vg/kg dose cohort); 1 of the events in the $2x10^{12}$ vg/kg dose was reported as a SAE. All of them were resolved with steroid intervention. The cause of a transient and asymptomatic hepatic transaminase elevation (i.e., elevated ALT and/or AST) observed in earlier liver-directed AAV-mediated gene transfer clinical trials has not been established. However, some individuals have developed dose-dependent T cell responses to AAV capsids, in the context of elevation in transaminases, and the presumptive mechanism is a memory $CD8^+$ T cell response to peptides derived from the AAV capsid of the vector (Mingozzi, 2007). [redacted]

In a recent AAV-based gene therapy Phase 1/2 clinical trial investigating AT132 for X-linked myotubular myopathy (XLMTM, NCT03199469 [ASPIRO]), 3 child participants who were older age and heavier weight, had pre-existing liver disease, and were from the highest tested dose cohort of $3x10^{14}$ vg/kg AT132 experienced SAEs of hyperbilirubinemia. All 3 SAEs

resulted in fatal outcomes caused by progressive liver dysfunction followed by bacterial infections, sepsis, and GI bleeding. The high AT132 dose of $3x10^{14}$ vg/kg is the highest tested dose for any AAV-based gene therapy to date. Among the 6 child participants treated at $1x10^{14}$ vg/kg, including 4 with a previous history of hepatobiliary disease, none have yet developed hepatic SAEs despite being years out from treatment (High-dose AAV, 2020; Audentes Therapeutics, 2020a; Audentes Therapeutics, 2020b). Note that the patient population (adults in the SPK-8016-101 study versus children in the ASPIRO study) and disease severity (Hemophilia A is a less severe disease than XLMTM) are quite different between Study SPK-8016-101 and the ASPIRO study. Moreover, individuals with medically significant underlying liver disease will be excluded from participating in the SPK-8016-101 study and the starting dose that was tested in the study ($5x10^{11}$ vg/kg) is 3 orders of magnitude lower.

2.3.1.4 Anti-AAV Neutralizing Antibody Development

As is expected after systemic administration of AAV vectors, all 4 participants (100%) in study SPK-8016-101 who were treated with SPK-8016 developed neutralizing antibodies to the AAV capsid. This risk seems to have no immediate impact on the expression of FVIII produced by the current AAV gene therapy, but development of neutralizing antibodies to AAV could potentially preclude the chance of a participant receiving another AAV gene therapy in the future.

2.3.1.5 Bleeding Episodes

The proposed dose levels of SPK-8016 may not be sufficient to raise the vector-derived FVIII:C levels to a therapeutic level for controlling and preventing bleeding episodes when the participants halt their prophylactic (factor or non-factor replacement) treatment after vector administration. Clinical experience suggests most patients can stop routine prophylaxis approximately 2 to 4 weeks after receiving SPK-8016 based on the expression of FVIII from the transgene. Participants will be advised to treat their bleeding events with their usual FVIII products during the follow-up observation period. Although bleeding (the nature of the disease) is not considered an adverse event, participants will record the dates of bleeding events, the type and location of the bleed, and the FVIII product and dosage used to treat the bleeding episode in the infusion log (Section 13).

2.3.1.6 Possible Side Effects from Corticosteroids

To mitigate the potential for harm due to cytotoxic T-lymphocyte (CTL) response against the capsid and to maintain endogenous FVIII expression, corticosteroids are allowed in the current protocol for participants who develop elevated transaminases and/or declining FVIII activity. Corticosteroids for the interruption of autoimmune-mediated inflammation most commonly have been given as daily oral therapy (e.g., prednisone or prednisolone) or as pulses of intravenously dosed [redacted] (Gordon, 2018).

Participants with elevated hepatic transaminases will be monitored closely to minimize the risk of side effects, to utilize the lowest effective dose and to shorten the duration of the corticosteroid therapies by tapering as soon as there is evidence of resolution of hepatic transaminase elevation and stabilization of FVIII. The disappearance of capsid-specific T cells, as measured by ELISpots, from the peripheral blood is an exploratory assay and may or may not

signal an immune response. While on corticosteroids, participants will also be monitored for side effects including opportunistic infections.

Corticosteroids such as [redacted], prednisolone, and prednisone have a number of well-described side effects including: hypertension, edema and swelling, tachycardia, congestive cardiac insufficiency, alterations in serum electrolytes, hyperglycemia, pain, increased blood urea nitrogen (BUN) and creatinine, osteoporosis and avascular necrosis of bone, decreased resistance to infection, cataract development, dizziness, trembling, emotional instability, insomnia, nausea, vomiting, weight gain, and elevated intraocular pressure.

Populations at special risk for side effects from prolonged and/or high dose corticosteroid use include individuals with pre-existent osteoporosis (in particular vertebral osteoporosis), brittle diabetes, labile hypertension, obesity, and emotional instability. The use of alternative or combined immune modulating agents may allow lower exposure to corticosteroids (also discussed below in Section 2.3.1.7).

Steroid-associated and nonserious adverse events (e.g., cushingoid appearance, candida infection, swelling face, tooth infection, increased blood glucose, and fatigue) have been reported in 3 of the enrolled participants in study SPK-8016-101 due to excessive long-term use of corticosteroids. The planned [redacted] in future enrolled participants are aimed to reduce the need for corticosteroids to avoid these side effects.

2.3.1.7 Risks Associated with Additional Immune Modulating Agents

The potential side effects of corticosteroids increase in prevalence as treatment is prolonged. For this reason, inflammatory conditions that respond to corticosteroid monotherapy are frequently treated instead with combined immune modulating agents to achieve anti-inflammatory efficacy while limiting the quantity and/or duration of corticosteroid exposure. An example of such a "steroid-sparing approach" that has been clinically validated is provided in the guidelines for the treatment of autoimmune hepatitis recommended by the American Association for the Study of Liver Disease (AASLD) and by the European Association for the Study of the Liver (EASL). Both the AASLD and the EASL provide recommendations [redacted] to prednisone/prednisolone therapy to provide therapeutic benefit while using lower steroid doses and/or to successfully taper off corticosteroids. Additional immune modulating agents, including [redacted], have been used to overcome suboptimal response to corticosteroid monotherapy or [redacted] combined therapy in the setting of immune-mediated hepatic inflammation. Participants who are treated with [redacted], [redacted], or other immune suppressive agents will be monitored closely as described above (Section 2.3.1.6) to assess side effects and to enable use of the lowest dose and shortest course of immune modulating agents.

Use of any of these drugs with AAV, concomitant medications and/or other immunomodulatory drugs may increase the risk of hepatotoxicity.

Use of any immunosuppressants may increase the risk of infection. This may include community acquired viruses such as influenza and COVID-19, as well as opportunistic infections.

Immunosuppressive therapy may enhance adverse/toxic effects of live vaccines and may diminish the protective effect of vaccines. Due to the potential that trial participants may receive

an immunosuppressive medication during the SPK-8016-101 trial, it is suggested that the Investigator and participant review and complete all age-appropriate vaccinations during the screening period and at least 4 weeks prior to the planned day of administration of SPK-8016. If that patient is currently receiving immunosuppressive medication, timing of vaccinations could be adjusted around the patient's course of immune suppressant treatment.

2.3.1.7.1 [redacted]

[redacted]

2.3.1.7.2 [redacted]

[redacted]

[redacted]

[redacted]

2.3.1.7.3 [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.3.1.7.4 [redacted]

[redacted]

[redacted]

[redacted]

2.3.1.7.5 Immune Suppressive Medications and Risk of Infections, including COVID-19 Risk

Immune suppressive medications including [redacted] used alone or in combination, may increase the risk of bacterial, mycobacterial, fungal and viral infection. This may include community acquired viruses such as influenza and COVID-19 as well as opportunistic infections. Extensive information regarding the risk of contracting COVID-19 and the clinical course of COVID-19 for individuals taking immune suppressant medications and individuals with immune suppression is currently unknown (Guan, 2020; Richter, 2016; Ni, 2019; Bello, 2012; Russell, 2020a; Russell, 2020b; Favalli, 2020). The chronic use of corticosteroids and biological immune suppressive agents has been associated with increased rates of influenza infection (an example of epidemic pneumonia), so increased vigilance regarding diagnosis is particularly important because symptoms of infection, including fever, may be masked by the medications (Favalli, 2020). The clinical course of COVID-19 infection including morbidity and mortality for individuals taking immune suppressant medication is the subject of prospective investigation in clinical trials (Richter, 2016; Russell, 2020a; Russell, 2020b; Favalli, 2020). Corticosteroid use in the treatment of the Acute Respiratory Distress Syndrome (ARDS) of influenza has been associated with increased mortality and increased morbidity including secondary bacterial and fungal infections (Ni, 2019; Favalli, 2020; Russell, 2020a; Russell, 2020b). Although corticosteroids were employed as a

potential supportive therapy in the treatment of the ARDS associated with coronavirus outbreaks, SARS-CoV and Middle East respiratory syndrome-related coronavirus (MERS-CoV), clearance of the virus and steroid-associated complications resulted, without improved mortality. Several clinical trials are currently examining the potential therapeutic value of [redacted] and other biological immune suppressive drugs in suppressing the cytokine release syndrome caused by SARS-CoV2 that drives ARDS, however, results are not yet available to guide current management (Russell, 2020a; Russell, 2020b).

As clinical evidence accrues, it is advised to consider participants using immune suppressive medication in the SPK-8016 clinical trial program to be at increased risk of infection (clinically susceptible) including pneumonia infection with community acquired influenza and COVID-19. Increased vigilance for signs and symptoms consistent with a diagnosis of COVID-19 is warranted because symptoms of infection, including fever, may be less clinically evident (Favalli, 2020) while taking immune suppressive medication. Careful clinical management should include instruction regarding COVID-19 preventive measures, e.g., social distancing and hand washing hygiene (Public Health England, 2020; National Health Service, 2020). Investigators along with their SPK-8016-101 clinical trial participants should consider individualized approaches to social/behavioral interactions as well as clinical visits and treatments consistent with national, local, and institutional COVID-19 prevention guidance (US Food and Drug Administration, 2020).

2.3.2 Potential Benefits

The main purpose of study SPK-8016-101 is to evaluate the safety of SPK-8016 at 3 or 4 different dose levels. It is not known if higher dose levels will raise the activity level of FVIII in humans, but, based on prior clinical experience in hemophilia B and non-clinical experience in NHPs with SPK-8016, the dose levels to be tested may result in detectable FVIII:C. Current commercially available FVIII concentrates exhibit an in vivo half-life (ranging from 10.8 to 19.7 hours) that requires IV injections approximately every 2 to 3.5 days for effective prophylactic treatment (NHF-MASAC Recommendations Document #240). SPK-8016 has the potential to drastically reduce this frequency of factor administration in the prevention of bleeding episodes. Additionally, data generated in this study will generate important scientific insights about rAAV-mediated gene transfer for hemophilia.

2.4 Rationale

Published data from Nathwani and colleagues (Nathwani, 2014) of an AAV8-mediated self-complementary hFIX gene transfer trial for severe hemophilia B demonstrated long-term expression of FIX activity levels with mean (±SD) of 5.1 ±1.7% of normal in 6 participants at the highest dose level of $2x10^{12}$ vg/kg, over a median period of 3.2 years after vector administration. No vector- and procedure-related safety concerns were observed in the initial study or the 3-year LTFU. Four of these 6 participants developed an asymptomatic rise in hepatic transaminases between weeks 7 and 10 after vector administration, which required a short, approximately 8-week tapering course of prednisolone therapy to prevent loss of FIX transgene expression. The recent results from an AAV5-mediated [redacted] gene transfer trial for severe hemophilia A demonstrated FVIII activity levels that were sub-therapeutic (0.6% of normal) over 34 weeks in

1 participant dosed at $6x10^{12}$ vg/kg (low dose-level), levels of 2.3% of normal over 26 weeks in 1 participant dosed at $2x10^{13}$ vg/kg (middle dose-level), and levels of 5 - 271% of normal in 7 participants at $6x10^{13}$ vg/kg (high dose-level) in the first year after vector infusion (Rangarajan, 2017). However, longer-term follow-up has shown a gradual decline in FVIII levels in the circulation. Thus, at year 1, 6 out of 7 participants showed FVIII within the normal range, and 1 out of 7 displayed mild hemophilia. At year 3, 1 out of 7 remains in the normal range, 5 out of 7 are in the mild hemophilia range, and 1 out of 7 is in the moderately severe hemophilia range. At the year 3 median of 19.9%, half the participants are either at or approaching expression levels associated with bleeding events. In that trial, in order to avoid the asymptomatic rise in hepatic transaminases concomitant with the loss of transgene FVIII activity levels, all 7 participants receiving the highest dose of BMN-270 received a course of steroid therapy. Six participants received prophylactic corticosteroid regimens starting 3 weeks after vector administration and had exposure from 15 weeks to at least 35 weeks in 1 participant (Pasi, 2020).

In the Sponsor's similar gene therapy trial SPK-8011-101, 17 participants have been dosed with SPK-8011 at the following dose levels: $5x10^{11}$, $1x10^{12}$, $2 x 10^{12}$, and $1.5x10^{12}$ vg/kg. Two participants in the low dose cohort, $5x10^{11}$ vg/kg, did not mount an immune response and have sustained FVIII expression of ~5% of normal. Three participants in the second dose cohort, $1.0x10^{12}$ vg/kg, express FVIII ~ 4-23% of normal, with 2 of these participants receiving corticosteroids post vector infusion for a declining FVIII level. In the $2x10^{12}$ vg/kg dose cohort, 7 out of 9 participants demonstrate FVIII expression ranging from ~ 3-22% of normal. The 2 participants who lost expression have returned to prophylaxis uneventfully. Prophylactic corticosteroids were explored in 2 participants at $2x10^{12}$ vg/kg. In both, early initiation of corticosteroids resulted in a transient supraphysiologic increase in FVIII levels to greater than 200% of normal. Based on discussions with the study's independent DMC, an intermediate dose cohort ($1.5x10^{12}$ vg/kg) was initiated. Reduction of the vector genome dose was successful in avoiding supraphysiologic levels of FVIII, but in all participants where corticosteroids have been started prior to 6 weeks post vector infusion, tapering off has proven difficult, and consequently, prolonged courses have been required, with associated steroid related side effects. In these participants, steroid sparing agents have allowed a taper of the prednisone. Because of the need for a prolonged steroid taper when steroids are administered beginning at 4 weeks, which is not observed with later initiation of steroids, prophylactic steroids will be avoided going forward. Optimal vector dose and immune suppression regimens, including alternatives to daily oral steroid use, are being explored to optimize predictable, safe, efficacious and durable FVIII expression (George, 2019).

A vector that can safely and consistently achieve sustained high levels of FVIII activity and potentially eliminate spontaneous hemarthrosis are the goals for the incorporation of gene transfer into hemophilia care.

The objective of this study is to determine the safety, tolerability, and efficacy as evidenced by prevention of bleeds and level of FVIII expression of a single IV infusion of SPK-8016 in individuals with hemophilia A.

2.5 Rationale for Dose and Schedule Selection

[redacted] In non-clinical studies, conducted in both NHPs and in cell culture, SPK-8016 resulted in increased expression of B domain-deleted FVIII, ranging from 2.6- to 4.4-fold higher in *in vivo* experiments, and 3.7- to 6.6-fold higher in *in vitro* experiments. With this promising nonclinical data support, the Sponsor hypothesizes that SPK-8016 will be able to deliver the lowest dose possible with the lowest toxicity and with sufficient FVIII expression for hemophilia A patients.

Table 2 provides Factor VIII plateau levels in Phase 1 Study SPK-8011-101 from 5 participants infused at doses of $5x10^{11}$ vg/kg or $1x10^{12}$ vg/kg. All factor levels are measured by one stage clotting assay in the central laboratory. A chromogenic assay using the Biophen FVIII:C chromogenic assay (Hyphen BioMed) gives levels that are similar to or lower than the one stage assay. Plateau levels are determined by averaging all values obtained over the period from 12 weeks following vector infusion until the most recent value (range 142-182 weeks).

Table 2: Factor VIII Plateau Levels in Study SPK-8011-101

Participant number	Dose	Date infused	Plateau FVIII by one stage clotting assay central lab
[redacted]	$5x10^{11}$ vg/kg	[redacted]	11%
	$5x10^{11}$ vg/kg		7.3%
	$1x10^{12}$ vg/kg		4.4%
	$1x10^{12}$ vg/kg		9.9%
	$1x10^{12}$ vg/kg		22%

Of note, the range is similar for the 2 doses, which is not surprising since there is only a 2-fold difference in dose. However, the data at the 2 doses provides evidence for the estimate of levels of expression that can be obtained at the $5x10^{11}$ vg/kg dose in the SPK-8016-101 study. The highest-fold increase from the *in vitro* experiments comparing SPK-8011 and SPK-8016, and the higher plateau level obtained at the $5x10^{11}$ vg/kg dose with SPK-8011 are used as references to predict that the plateau FVIII level would be approximately 7 x 11% = 77%, comfortably within a normal range.

Thus, planned dose levels include $5x10^{11}$, $1x10^{12}$, and $2x10^{12}$ vg/kg of SPK-8016.

At the starting clinical dose of $5x10^{11}$ vg/kg, FVIII activity levels around 6% of normal were achieved in 3 participants. One participant has FVIII expression of >20% one-year post SPK-8016 infusion. Although the FVIII levels achieved have reduced the annualized bleeding rates for these participants, FVIII expression remains below the normal range. Optimal vector dose and immune suppression regimens are needed.

Therapeutic rAAV-based gene transfer is hampered in individuals who develop neutralizing antibodies against AAV capsids. Even if switching to a different vector serotype, participants who develop these are unlikely to qualify for future gene transfer clinical studies. For this reason, the Sponsor proposed the starting dose level of $5x10^{11}$ vg/kg that could provide a reasonable therapeutic level while offering an ample safety margin for the participants.

Safety of these dose levels is supported by previous and ongoing human clinical trials of [redacted], as well as the NHP studies with SPK-8011 in which dose levels up to $2x10^{13}$ vg/kg were infused without evidence of adverse effects. Notably, the NHP dose level is ~40-fold higher than the proposed starting dose level of $5x10^{11}$ vg/kg in this clinical study.

The Sponsor has previously demonstrated that the presence of an excess of empty capsids may adsorb low-level and non-neutralizing antibodies, permitting liver transduction after peripheral vector infusion even in their presence (Mingozzi, 2013b). This hypothesis is consistent with the clinical data from previous AAV-mediated hemophilia B gene transfer trials, in which the presence of empty capsids in the formulation correlated well with higher expression levels at low vector doses (Monahan, 2015; Nathwani, 2014). [redacted]

3 OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

Primary objectives for this study are:

- To evaluate the safety and tolerability of SPK-8016.
- To evaluate the efficacy as evidenced by prevention of bleeds and level of FVIII expression with SPK-8016.

3.2 Secondary Objectives

Secondary objectives for this study are:

- To evaluate additional parameters associated with SPK-8016 directed FVIII expression and vector shedding.
- To characterize the immune response to the vector and transgene product.

3.3 Primary Endpoints

The primary endpoints include the following:

- For safety and tolerability:
  - Incidence of adverse events, including clinically significant abnormal laboratory values.
  - Occurrence of hepatic transaminase elevation requiring immunosuppression.
- For efficacy:
  - Peak and steady-state FVIII activity levels assessed by coagulation clotting assays. In this study, steady-state levels are based on FVIII:C measurements starting 12 weeks post vector administration and without the use of exogenous FVIII products since vector administration.
  - Number of bleeding events (spontaneous and traumatic) since vector administration.
  - Number of FVIII infusions since vector administration.

3.4 Secondary Endpoints

The secondary endpoints include the following:

- Additional kinetic assessments including, but not limited to:
  - Time to achieve steady-state FVIII activity levels;
  - Vector-shedding of SPK-8016 in bodily fluids.
- Incidence of immune responses to AAV capsid protein and BDD-hFVIII transgene.

3.5 Exploratory Endpoints

The exploratory endpoints will assess changes from baseline in the following:

36. Joint assessments:

- Number of target joints
- Hemophilia Joint Health score

37. Activities assessments:

- Hemophilia Activities List
- Change in Level of Activity questionnaire

38. Quality-of-life assessments:

- Haem-A-QoL questionnaire
- Euro Quality-of-Life Five Dimensions (EQ-5D-5L) questionnaire

39. Health-economic parameters including, but not limited to, collection of information on the following:

- Number of hospitalizations (excluding pre-planned hospitalizations documented at screening)
- Number of hospitalization days
- Number of emergency room visits
- Number of physician visits, excluding study visits
- Number of days off school or work

40. Exploratory inflammatory profiling of plasma and immune function gene expression of PBMCs after vector administration (ELISpot, and other exploratory biomarkers)

4 STUDY DESIGN

4.1 Overall Design

SPK-8016-101 is a Phase 1/2a, open-label, non-randomized, dose escalation study to evaluate the safety, tolerability, and efficacy of a single IV infusion of SPK-8016 in men with clinically severe hemophilia A, and no measurable inhibitor against FVIII. A maximum of 40 evaluable (i.e., dosed) participants will be dosed with a single IV infusion of SPK-8016.

4.1.1 Sequence of Enrollment

The following 2 staggering strategies are used in this study based on the recommendation from *The Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products* (Center for Biologics Evaluation and Research, Office of Cellular, Tissue, and Gene Therapies, Food and Drug Administration; US Dept. of Health and Human Services 2015):

- The first 2 participants in each dose-level ($5x10^{11}$ vg/kg, $1x10^{12}$, $2x10^{12}$ vg/kg) will be infused with SPK-8016 at least 6 weeks apart to mitigate acute safety risk; and
- An independent DMC will review at least 6 weeks of follow-up data from a minimum of 2 participants who have received SPK-8016 at a given dose level prior to dosing the first participant in the next dose level.

After escalation through the first 3 planned dose levels, a fourth dose level may be explored in consultation with the DMC. Additionally, the Sponsor may decide to further expand the starting dose cohort ($5x10^{11}$ vg/kg), the middle dose cohort ($1x10^{12}$ vg/kg), or the high dose ($2x10^{12}$ vg/kg) to better evaluate the safety, efficacy, and variability of response within a given cohort. Any decision to add a fourth dose level or further expand one of the existing protocol-defined dose levels will be made in consultation with the DMC.

The dose level for Cohorts 1 & 2 will begin at $1x10^{12}$ to explore immunomodulation.

Cohort 1 will utilize [redacted] should a steroid sparing regimen be indicated, based on the clinical judgement of the Investigator and in consultation with the Sponsor. The steroid sparing approach will include consideration of individual risk including potential toxicity that may result from interactions with concomitant medications.

[redacted] The clinical course of these 2 participants will be monitored including findings potentially consistent with a cellular immune response targeting transduced hepatocytes (e.g., acute decrease in plasma factor VIII activity and/or acute increase in liver ALT transaminase). The clinical course of these first 2 participants who receive [redacted] will be reviewed by the Sponsor in consultation

with the DMC, including any additional immune monitoring parameters that are available from among the Immune Profiling assays (e.g., serum cytokines, acute phase reactants e.g., CRP, fibrinogen). Based upon the Sponsor and DMC review of clinical observations, a decision will be reached whether to allow [redacted] administered up to 4 weeks after vector infusion. In any event, [redacted] will not be administered later than 4 weeks following vector administration, due to concern that incidence of [redacted]-related transaminitis may increase with repeat dosing, which could in turn confound the effort to monitor vector-associated hepatocyte loss (which is most likely to occur 6 weeks or later following rAAV dosing). Due to the same safety consideration, a second dose of [redacted] will not be administered to any individual if the participant's current ALT is ≥ 1.5 x the participant's baseline ALT value (assayed prior to vector administration) or greater than the Upper Limit of Normal for the ALT (whichever of these 2 values is lower). Therefore, following the review of the data from the first 2 participants who receive a single Day 0 dose of [redacted], the criteria that would be considered a potential trigger for a second dose of [redacted] are: decline in factor VIII activity (e.g., decline of >30-40% of FVIII activity over a period of ≤ 1 week) with or without AAV IFN-gamma ELISpot positive. In addition, the presence of any of the following clinical observations, probably or possibly a complication of the initial dose of [redacted], are a contraindication to a second dose of [redacted]:

a. Hypersensitivity/Anaphylaxis in response to [redacted]
b. Transaminitis. Do not give a second dose of [redacted] if the ALT greater than the Upper Limit of the Normal Range or greater than 1.5 times the study subject's baseline ALT value (if the latter degree of elevation remains within normal limits).
c. Neutropenia. Do not give a second dose of [redacted] if the ANC <2000 per mm^2
d. Thrombocytopenia. Do not give second dose of [redacted] if platelet count is <100,000 cells/µL
e. Hypofibrinogenemia. Do not give a second dose of [redacted] if fibrinogen antigen is <180 mg/dl

Cohort	Number of Participants	SPK-8016 Dose	Immunomodulatory Regimen
1	3-5	$1x10^{12}$ vg/kg $2x10^{12}$ vg/kg	[redacted]
2	3-5	$1x10^{12}$ vg/kg $2x10^{12}$ vg/kg	[redacted]

[redacted].

Enrollment will begin in either Cohort 1 or 2 at a dose of 1 x 10^{12} vg/kg then alternate between cohorts. A stagger of 4 weeks will occur between the first and second participants in Cohort 2. If in the opinion of the Investigator and Sponsor, there are individual or site-specific circumstances that might preclude enrollment in either cohort in an otherwise eligible participant, enrollment in the alternate Cohort may be considered (i.e., HIV status, timely access for IV steroid administration based on geographic location). The Sponsor has a similar investigational gene therapy, SPK-8011-101, evaluating immunomodulation prior to vector infusion (NCT03003533).

These data will be informative to SPK-8016 enrollment and shared with the DMC. If safety signals are observed in any cohort, the Sponsor in consultation with the DMC will decide whether and how the safety findings will impact future cohort assignment.

4.1.1.1 Dose-Level and Cohort Expansion

Dose-cohort expansion may occur under the following scenarios:

Dose Level Expansion **-** After 2 participants are dosed in a given dose level, it may be possible to expand a cohort to up to 10 participants if there is evidence of FVIII:C increases above 5% of normal in either participant by Week 4 post vector-administration. This initial expansion will provide additional information about variability of response within the same dose level.

New Cohort Expansion - After 2 participants are dosed in a cohort, an initial expansion of up to 3 participants may occur and, thereafter, an additional expansion for up to a total of 10 participants may occur. The additional expansion may occur in 1 or more cohort(s). For Cohort 2 of the 1 x 10^{12} dose, the first 2 participants will be infused at least 4 weeks apart.

If either of the immunomodulatory regimens is deemed clinically superior and if agreed to by the DMC, the cohort evaluating the less effective regimen will be closed to enrollment.

Dose Escalation

The decision to dose-escalate will be made by the Sponsor in consultation with the DMC to ensure safety.

Dose escalation to the next dose level may occur under the following scenarios, provided there are no safety concerns after at least 6 weeks of follow-up data have been reviewed by the DMC. Dose escalation through the first 3 dose levels ($5.0x10^{11}$ vg/kg, $1.0x10^{12}$, $2.0x10^{12}$ vg/kg) may occur in, but is not restricted to, the following scenarios.

Dose escalation may be considered if:

1. If **neither of the first 2 participants** achieve FVIII:C above 5% of normal by Week 6 post vector-administration; or
2. If **at least 2 participants** in a given cohort achieve FVIII:C ≤ 40% of normal by Week 6 post vector-administration; or
3. If **3 participants** in a given cohort achieve 'steady-state' FVIII:C ≤ 50% of normal.
4. There will be **no** dose escalation if at least 3 participants in any dose level achieve steady-state FVIII:C > 80% of normal. Steady-state levels are based on at least 2 separate FVIII:C measurements, at least 2 weeks apart, starting 8-12 weeks post vector administration while not receiving daily corticosteroid therapy and without use of exogenous FVIII products since vector administration.

Corticosteroid therapy may increase the transgene expression from the SPK-8016 expression cassette, similar to SPK-8011, as observed in both animal models and in our clinical observations using both early prophylactic and the reactive application of corticosteroids. In SPK-8011, this effect appears to occur in a dose-dependent fashion; in clinical observation the effect has appeared somewhat greater with initiation of daily oral corticosteroids in a prophylactic fashion in the early weeks after vector delivery when compared to reactive corticosteroids given at later time points. The Primary Endpoint for efficacy for this study is the FVIII activity at steady state,

which Spark does not interpret while any subject remains on corticosteroid treatment. Instead, expression level of steady-state FVIII:C as an efficacy endpoint is evaluated after daily oral corticosteroids or pulse steroids have been discontinued for at least 2 weeks. Note that if the concomitant use of steroids is to have an effect to artifactually increase the FVIII expression to a level greater than the eventual steady state expression (after discontinuation of steroids), this would not trigger an inappropriate escalation of the SPK-8016 dose (which would be a safety concern) but instead might result in an expansion of the present dose cohort or a delay in the decision to dose escalate until after the observation of steady state while off steroid therapy.

[redacted]

In consultation with the DMC, further dose exploration may be considered if effective immunomodulation has been demonstrated in any cohort and/or FVIII expression is less than 80% of normal 12 weeks following gene transfer in at least 2 participants.

4.1.2 FVIII Incremental Recovery

If FVIII levels resulting from endogenous FVIII production from transduced hepatocytes begin to decline without evidence of elevation of transaminases, the participant will be evaluated for evidence of anti-FVIII antibody formation. This evaluation will include FVIII inhibitor assays and FVIII incremental recovery with blood sample collection consistent with the Investigator's clinical practice after receiving 50 IU/kg of FVIII product. Blood sampling will be done at appropriate time-points to enable acceptable determination of PK parameters (Iorio, 2016).

4.1.3 Corticosteroids

Based on observation and experience from earlier clinical studies of liver-directed AAV gene transfer, including the SJ-UCL trial (Nathwani, 2011b; Nathwani, 2014), the Sponsor's earlier clinical studies [redacted], and the Baxalta BAX 335 trial (NCT#01687608), participants may develop an immune response to the vector capsid, as evidenced by a transient rise in transaminases (AST and/or ALT) and/or a loss in FVIII activity in the peripheral blood, as measured by IFN-gamma ELISpot (Manno, 2006; Mingozzi, 2007; Nathwani, 2011b). In the Sponsors' similar gene therapy trial, SPK-8011, 12 participants have required corticosteroids following vector infusion. Two participants in the low dose cohort, $5x10^{11}$ vg/kg, did not mount an immune response. Two of the 3 participants in the second dose cohort, $1.0x10^{12}$ vg/kg, received corticosteroids starting at 5 and 11-weeks post vector infusion; a declining FVII level was the trigger. Of the 7 initial participants treated in the third dose cohort with $2x10^{12}$ vg/kg SPK-8011, 5 required corticosteroids, starting at 6 to 11-weeks post vector infusion for 1 or more of the following triggers: declining FVIII levels, rise in ALT above participant baseline or elevated IFN-ɣ ELISpots to AAV capsid. Steroid initiation normalized

ALT levels and extinguished the ELISpot signal in all cases. Two of the 5 participants who received corticosteroids in the $2x10^{12}$ vg/kg dose group, however, showed loss of transgene expression likely due to the immune response. Both of these individuals eventually returned to hemophilia prophylaxis regimens, one with recombinant FVIII, the other with emicizumab. Given that 5 of 7 participants in the $2x10^{12}$ vg/kg group required corticosteroids, including 2 participants who lost FVIII expression, the institution of a consistent, standardized short course of prophylactic corticosteroids appeared warranted in an attempt to pre-emptively manage potential immune responses. In the first 2 participants administered SPK-8011 at $2x10^{12}$ vg/kg using prophylactic oral corticosteroids (1 mg/kg or 60-80 mg), the first was started on corticosteroids on Day 27 due to falling FVIII and the second was started on Day 26 due to rising ALT and decreasing FVIII expression. In both of these participants, early initiation of corticosteroids resulted in a transient supraphysiologic increase in FVIII levels to greater than 200%. An intermediate dose cohort ($1.5x10^{12}$ vg/kg) was initiated. Reduction of the vector genome dose was successful in terms of avoiding supraphysiologic levels of FVIII upon initiation of corticosteroids, but in all participants where corticosteroids have been started prior to 6 weeks post vector infusion, tapering off has been challenging, and consequently, prolonged courses have been required, with associated steroid related side effects. [redacted] Because of the prolonged steroid taper when steroids were administered beginning at 4 weeks, which was not observed with later initiation of steroids, prophylactic steroids will be avoided going forward.

Based on discussions with expert immunology consultants, the Sponsor proposes to again investigate administration of steroids only in response to one of the previously described triggers, i.e., declining FVIII levels or rising transaminases, [redacted] Oral corticosteroids may be utilized, if clinically indicated. ELISpot is an exploratory assay and not predictive as a trigger for immunomodulation.

In this SPK-8016-101 study, 4 participants have received SPK-8016 at dose level $5x10^{11}$ vg/kg. Three of the 4 participants developed an apparent immune response to the AAV capsid, characterized by transient elevations in liver transaminases and a decrease in FVIII expression, and/or persistent positive IFN-γ ELISpot results to the [redacted], which have prompted initiation of corticosteroid therapy (prednisone). Prednisone was started week 3-6 post infusion. Prolonged tapering of daily oral corticosteroids has been required in all participants (total courses of 43-48 weeks prednisone) to avoid and treat late recurrences of apparent hepatocyte-targeted immune response. [redacted]

[redacted].

Significant elevations of transaminases (≥5-10x ULN) will be treated as a safety issue and appropriate medical care will be instituted according to the standard of care of the medical facility involved.

Based on the data observed to date in this trial as well as in Hemophilia B trials (Manno, 2006; Nathwani, 2014), a course of corticosteroids may be initiated in participants, depending on the clinical judgement of the Investigator and in consultation with the Sponsor, after a trigger is identified in the transaminases or FVIII post vector transfusion and may be continued in a pulse or tapering regimen, IV and/or oral, until clinical concern of hepatic inflammation is resolved and per Investigator/Sponsor discretion. The ELISpot assay is considered exploratory and not a predictive trigger for reactive immunomodulation.

4.1.3.1 Corticosteroid Regimen

If transaminases become elevated or the FVIII declines, [redacted]

[redacted]

[redacted]. Treatment will continue until resolution of the immune response occurs and may require the use of additional immunomodulatory agents. Daily oral corticosteroids given in a tapering dose regimen may be utilized instead of [redacted] corticosteroids, if in the estimation of the Investigator an oral dosing regimen is clinically indicated. The Investigator in collaboration with the Sponsor will have flexibility in implementing the corticosteroid regimen, since the exact regimen and course will depend on clinical circumstances.

If reactive corticosteroids [redacted] or oral dosing) are triggered by an apparent immune response, additional blood samples for "Immune Profiling" (cytokines) and "PAX gene" should be collected 1) prior to the initiation of corticosteroids (if this is possible without delaying the initiation of corticosteroid therapy) and 2) at 1 week (± 2 days) and at 2 weeks (± 2 days) following the first dose of corticosteroids. At the discretion of the Investigator, nucleic acid amplification testing (NAAT) for infection with SARS-CoV-2 (the infectious agent in COVID-19) may be performed at this time for individuals who do not have symptoms consistent with upper or lower respiratory tract infection or COVID-19. NAAT testing should be performed at

this time for individuals with any of the following symptoms: cough, shortness of breath or difficulty breathing, fever, chills, sore throat, new loss of taste or smell; new persistent/recurrent muscle pain or headache. For individuals who do not have any of these symptoms, the initiation of reactive corticosteroids should not be delayed pending results of NAAT testing. For individuals who do display any of these symptoms, initiation of corticosteroids may be delayed at the discretion of the Investigator until after results of NAAT testing are known. Note: The Infectious Disease Society of America panel suggests collecting nasopharyngeal, or mid-turbinate or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19.

4.1.3.2 Other Immunomodulatory Considerations

Prolonged courses of high dose oral corticosteroids are undesirable and associated with adverse events. In the AASLD and the EASL guidelines for the treatment of autoimmune hepatitis, an alternative regimen of corticosteroids and steroid sparing agents, [redacted], is outlined for use in individuals who cannot tolerate high doses of prednisone or are experiencing difficulty tapering. A steroid-sparing agent administered concurrently with lower (compared to prednisone monotherapy) and tapering doses of prednisone may be utilized based on individual clinical circumstances. Both [redacted] have been utilized as steroid-sparing agents in the most recent iteration of this study, to good effect in both cases, and are described below.

[redacted]

The ultimate determination of steady state FVIII expression and determination of expression above a threshold FVIII:C activity value are interpreted by evaluating serial FVIII:C activity over multiple timepoints examined after discontinuation of corticosteroids and of steroid-sparing immune suppressive agents.

4.1.3.3 [redacted]

[redacted]

4.1.3.4 Experience with Long Term Immunomodulation

There is extensive experience with corticosteroids and immunosuppressive regimens in hemophilia: first, as a maneuver to eradicate antibodies to factor VIII or IX, clinically termed inhibitors (Nilsson, 1976; Hultin, 1976; Hay, 2006) and second, in the setting of liver transplantation due to the high prevalence of hepatitis C among adults with haemophilia (Gordon, 1998; Wilde, 2002). Many individuals with hemophilia have been maintained on standard liver transplant immunosuppression regimens for years. Corticosteroids have been used to treat people with asthma, idiopathic thrombocytopenic purpura, organ injury from autoimmune diseases including autoimmune hepatitis and systemic lupus erythematosus nephritis and lupus CNS manifestations, and other medical conditions. For participants with severe hemophilia A, the benefit of long-term expression of a modest level of clotting factor far outweighs the risk of receiving a course of corticosteroids or other immunomodulatory agents.

The long-term side effects of the immunomodulatory drugs expected to be used in this study are well-characterized. Participants who develop immune hepatitis will be monitored closely to minimize the risk of the side effects. Pulse dosing of steroids has been shown to be efficacious in reducing an immediate immune response with a reduction in corticosteroid related adverse events (when compared to daily high dose oral corticosteroids) in several autoimmune diseases (Fanouriakis, 2019; Gordon, 2018; Wei, 2016). The use of oral prednisone in [redacted] may reduce the reactivation of the inflammatory process or

rebound of the immune response; however, a standard regimen has not been determined (Gordon, 2018; Torres, 2018). To utilize the lowest effective dose and shorten the duration of the immunosuppressive therapies, tapering of the regimen will start as soon as there is evidence of resolution of hepatic transaminase elevation and disappearance from the peripheral blood of capsid-specific T cells. While on immunomodulatory regimens, participants will also be monitored for side effects, including opportunistic infections. Subjects should receive *Pneumocystis jiroveci* pneumonia prophylaxis while on immunosuppression. Antibiotics or other medications to minimize the risk of opportunistic infection may be prescribed at the discretion of the Investigator. All events related to the use of immunomodulatory drugs (e.g., hyperglycemia, weight gain, infections, acute exacerbation of hypertension or mood disorder, insomnia) will be recorded as AEs and attributed to immunomodulation, and medications may be prescribed at the discretion of the Investigator.

Individual participant recommendations regarding the use of alternative and/or additional immunomodulatory regimens or therapies (other than corticosteroids); e.g., [redacted] will be discussed with the Investigator(s) and the Sponsor and will involve a thorough review of the participants medical history, labs, concomitant medications, and clinical presentation. Depending on the agent selected, additional blood draws for therapeutic or laboratory monitoring and subsequent dosage adjustment may be required. The Investigator will discuss the risks and benefits of any immunomodulatory therapy with the participant and will consult with additional experts as needed (i.e., transplant surgeon, hospital pharmacist, infectious disease specialist, endocrinologist etc.).

Screening/Baseline: Participants who have provided consent will undergo the screening assessments described in Table 1 up to a maximum of 16 weeks prior to the infusion of SPK-8016. If there is no historical ≤1% FVIII activity levels documented by the certified laboratory, then the screening data must be generated documenting ≤2% FVIII activity at baseline.

Re-screening: Re-screening is permitted once, if the participant is found to be eligible for the study but the screening period is prolonged greater than 16 weeks. A participant may be re-screened into the study using the same participant ID; however, all screening assessments must be repeated.

4.1.4 Three to Seven Days Prior to Day 0

Participants in Cohorts 1 and 2 will require a laboratory assessment within 3 to 7 days prior to Day 0 to verify continued eligibility prior to starting immunomodulatory agents (i.e., [redacted]). This visit may be conducted by a remote provider. The study staff must contact the participant to verify and document no changes in the general health status prior to first dose of [redacted]

4.1.5 Day -2

[redacted]

4.1.6 Dosing Period (Days 0, 1)

Assessments and procedures to be performed on Dosing Day (Day 0) are described in Table 1. All participants will administer a prophylactic infusion of 50 IU/kg of their current FVIII concentrate the morning of Day 0. This may be self-administered and recorded in the subject infusion log. Site assistance is permitted for the FVIII infusion. On Day 0, participants in Cohort 2 will receive a dose of [redacted] intravenously over 60 minutes, with the [redacted] infusion to be completed at least 4 hours (up to 24 hours) prior to the start of vector administration. The [redacted] dose will not exceed [redacted] (Please see [redacted] prescribing information [Genentech, 2019]). Participants will receive a single IV infusion of SPK-8016 at the vector-administration center. The complete dose of SPK-8016 will be infused via infusion pump over approximately 60 minutes. Vital signs will be taken at various time-points from the start of infusion (see Table 1).

Blood samples for immune profiling will be collected pre-administration of [redacted] (3-7 Days Prior), and SPK-8016, and 30 (±2) minutes, 2 hours (± 10 minutes), 5 hours (± 10 minutes), and 24 (±1) hours from the completion of the SPK-8016 infusion. PAX Gene will be collected pre-administration of [redacted], pre-administration of SPK-8016, and 5 hours (± 10 minutes) and 24 (±1) hours from the completion of the SPK-8016 infusion. Additional blood samples, including FVIII activity levels, will also be collected pre-infusion of SPK-8016.

4.1.7 Follow-Up Observation Period

Participants who have received a dose of SPK-8016 will report to either the vector-administration center or follow-up center for follow-up evaluations, according to the protocol assessments for 52 (±2) weeks after the infusion of SPK-8016. During the follow-up observation period, a qualified/trained in-home service provider may be utilized, if needed, for remote-phlebotomy and sample-collection services during the visits which do not require physical examination. For participants in Cohorts 1 and 2, [redacted]

4.2 Study Duration, Enrollment and Number of Sites

4.2.1 Duration of Study Participation

The study will consist of the following periods

- Screening period (up to a maximum of 16 weeks)
- Study Intervention: Assignment into cohort with possible pre-infusion immunosuppressive medication or administration of oral immunosuppressive medication, and then study product infusion
- Follow-up observation period (52 [±2] weeks post-infusion of SPK-8016)

The total duration of the study for an individual participant is up to 68 weeks (including up to a maximum of 16 weeks of screening).

4.2.2 Total Number of Participants; Sites Projected and Geographic Regions

It is estimated that approximately 50 potential participants may be enrolled for a maximum of 40 evaluable (i.e., dosed) participants.

The study is planned to be conducted at approximately 15 study centers (vector-administration centers and/or follow-up centers) located worldwide.

4.3 Study Stopping Rules

The Sponsor may terminate this study at any time. The Sponsor, or designee, will notify the Investigator(s) and appropriate regulatory authorities if the study or any given study cohort is suspended, terminated, or completed. If the study or any given study cohort is suspended or terminated, the following instructions should be followed, unless the DMC and/or the Sponsor advise otherwise:

- Participants who have already received SPK-8016 will continue to maintain the protocol schedule;
- Participants who are in the screening phase of the study or study cohort but have not received SPK-8016 will wait for DMC recommendation. The scheduled date for SPK-8016 infusion may be postponed or cancelled.

Any of the following occurrences may result in suspension of further enrollment into the study or any given study cohort while the events are under investigation:

- Any SPK-8016 related death during the study;
- The development, in any participant, of SPK-8016 related Grade III-IV toxicity, including, but not limited to confirmed persistent FVIII inhibitor, allergic reaction (bronchospasm and anaphylaxis), excluding elevated transaminases;
- The development, in any participant, of SPK-8016 related (>10x ULN) elevated transaminases;
- The development, in any participant, of SPK-8016 related (≥2.5x ULN) elevated transaminases that fail to resolve to less than 2.5x ULN within 4 weeks on an immunomodulatory regimen;
- The occurrence, in any participant, of a medically important event that warrants further evaluation;
- Any occurrence of a malignancy at any point after vector infusion that is related to SPK-8016 or one of the immune modulating agents.

* *It is important to note that AAV vector-mediated insertional mutagenesis, if it should occur, is not likely to be observed in the initial year following gene transfer; therefore, the Sponsor intends to provide long-term safety monitoring of participants in a - LTFU study.*

In addition to halting enrollment, such an event will be handled as a SAE and reported in the timeframe according to Section 9.4.2. The DMC will review data relevant to the event and will receive information from the Sponsor and/or Investigator before providing appropriate recommendations. The event and the DMC's recommendation will be discussed with the U. S. Food & Drug Administration (FDA) and other regulatory authorities prior to re-initiation of

enrollment. All participants who were infused with the study drug will continue to comply with the follow-up schedule according to the protocol.

5 STUDY POPULATION

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 must meet all the following criteria at screening and prior to dosing of SPK-8016 (Day 0) to be eligible for the study:

1. Be able to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (PHI) in accordance with national and local privacy regulations
2. Be male and ≥18 years of age
3. Have hemophilia A with:
    a) < 1% (<1 IU/dL) endogenous FVIII activity levels as historically documented by a certified laboratory **or** screening data results; OR
    b) 1-2% (1-2 IU/dL) endogenous FVIII activity levels and >10 bleeding events per year (in the last 52 weeks prior to screening); OR
    c) 1-2% (1-2 IU/dL) endogenous FVIII activity levels and on prophylaxis
4. Have had >150 prior exposure days to any recombinant and/or plasma-derived FVIII protein products or cryoprecipitates based on historical data from medical records/history
5. Have no prior history of hypersensitivity or anaphylaxis associated with any FVIII or IV immunoglobulin administration
6. Have no measurable inhibitor against FVIII at screening (i.e., <0.6 Bethesda Units); no confirmed history of clinically significant FVIII inhibitor ***and*** no clinical signs or symptoms of decreased response to FVIII administration (Note: Family history of inhibitors is not exclusionary, nor is remote documentation (greater than 5 years) of a single measurement of Bethesda titer of >0.6 BU that is not accompanied by clinical evidence of failure to respond to infused FVIII concentrate)
7. Have acceptable laboratory values sampled at screening and reviewed prior to Day 0:
    a) Hemoglobin ≥11 g/dL;
    b) Platelets ≥100,000 cells/µL;
    c) AST, ALT, alkaline phosphatase < ULN;
    d) Bilirubin ≤1.5x ULN (Bilirubin levels above the laboratory's normal range are acceptable in individuals with a documented history or laboratory evidence of Gilbert's Disease);
    e) Creatinine ≤2.0 mg/dL;
    f) Absolute neutrophil count (ANC) ≥ 2000 per mm^3;

g) Fibrinogen antigen ≥ 180 mg/dL for participants in Cohort 2

8. Agree to use reliable barrier contraception after the administration of SPK-8016 until notified by the Investigator or designee.

5.2 Exclusion Criteria

Participants who meet any of the following criteria at screening or prior to dosing of SPK-8016 (Day 0) are not eligible for the study:

1. Have active hepatitis B or C. All participants must be screened for both active hepatitis B and C regardless of prior known history.
   a. **Screening for hepatitis B:** All participants must have a single sample collected at screening for each of the following tests: HBsAg (hepatitis B surface antigen), anti-HBc (total Hepatitis B core antibody), and HBV-DNA viral assay (nucleic acid test for hepatitis B virus DNA).
      i. A participant is not eligible if either HBsAg is positive or HBV-DNA is positive/detectable.
      ii. A participant is eligible if the anti-HBc is positive and both HBsAg and HBV-DNA are negative, as this would be consistent with a prior infection of hepatitis B. Anti-HBc must be obtained in all participants to discriminate between acute infection and possible reactivation of hepatitis B during the trial (e.g., in participants with no prior history of hepatitis B).
   b. **Screening for hepatitis C:** All participants, including those who have never been treated or who have completed anti-viral therapy for chronic hepatitis C, must have a single **HCV-RNA load assay (also referred to as a nucleic acid test [NAT] for HCV RNA)** at screening.
      i. A participant is not eligible if his HCV-RNA load assay is positive/detectable.
      ii. A participant treated with anti-viral therapy for chronic hepatitis C must have completed anti-viral therapy at least 6 months prior to screening and must have a negative HCV-RNA at the time of screening.
      iii. A participant with a documented or self-reported history of HCV must have a single negative HCV-RNA at the time of screening.
2. Are currently on antiviral therapy to treat their hepatitis B or C
3. Have significant underlying liver disease. A participant is not eligible with any of the following documented diagnoses, indicative of significant underlying liver disease:
   - Portal hypertension; *or*
   - Splenomegaly; *or*
   - Hepatic encephalopathy.

   Any participant without any of these pre-existing diagnoses must have the following performed at screening:

a. Serum albumin measurement. A participant is not eligible if the serum albumin level is below the lower limit of normal of the laboratory; ***and***
b. Diagnostic test for liver fibrosis (e.g., FibroScan, FibroTest/FibroSURE, or AST-to-Platelet Ratio Index [APRI]). A participant is not eligible if any of the following findings, which are indicative of fibrosis ≥ stage 3, are present:
   i. FibroScan score > 8.3 kPa units; *or*
   ii. FibroTest/FibroSURE > 0.48; *or*
   iii. APRI > 1

   If more than 1 diagnostic test result is available, then the FibroScan score will be used as the primary consideration for eligibility

4. Have serological evidence of HIV-1 or HIV-2 with CD4 counts ≤200/mm^3.
   a. Participants who are HIV-positive and stable, with an adequate CD4 count (>200/mm^3) and undetectable viral load (<50 gc/mL) , measured at screening, and who are on an antiretroviral drug regimen are eligible to enroll
5. Have [redacted] neutralizing antibody titers ≥1:1
6. Have a history of active cancer in the past 6 months, chronic infection, latent or active TB, uncontrolled immune disorder or other chronic disease that the Investigator and/or Sponsor consider to constitute an unacceptable risk
7. Have been dosed in a previous gene therapy research trial within the last 52 weeks or have participated in a clinical study with an investigational drug within the last 12 weeks prior to signing the informed consent
8. Have a history of diverticulitis, diverticulosis requiring antibiotic treatment, or chronic ulcerative lower G.I. disease that might predispose a patient to perforations
9. Have any concurrent clinically significant major disease (such as liver abnormalities, type I diabetes, uncontrolled hypertension, or vertebral compression) or any other condition such as active infections or COVID-19 or any other unspecified reasons that, in the opinion of the Investigator and/or Sponsor, makes the participant unsuitable for participation in the study.
   a. At the time of screening, the Investigator will consider the local geographic and institutional epidemiology of COVID-19 and other infectious pathogens when determining suitability of the participant for participation in the study, including considering the potential clinical relevance of additional screening
10. Have a planned surgical procedure in the next 12 months requiring FVIII prophylactic treatment
11. Are unable or unwilling to comply with the schedule of visits and study assessments described in the clinical protocol.

Participants who do not meet all of the enrollment inclusion criteria may not be enrolled. Any violations of these criteria must be reported in accordance with Sponsor and Internal Review Board (IRB) Policies and Procedures.

5.3 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently administered study drug. A minimal set of information will be collected for screen failures: demography, screen failure details, eligibility criteria, and, if applicable, any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened unless previous approval has been received from the Sponsor.

5.4 Enrollment of Participants

Each participant will be assigned a unique participant identification (ID) number after providing written informed consent. The participant ID number will be used to identify the participant for the duration of the study. Participant ID numbers will not be reassigned or reused. No participant may be dosed prior to obtaining the unique ID number. The Investigator must confirm and verify the inclusion/exclusion criteria, following the review of screening results from the laboratory and other documents.

5.5 Randomization

This is a non-randomized study; therefore, there is no randomization of patients.

5.6 Blinding Procedures

This is an open-label study; therefore, there is no study blinding.

6 STUDY PROCEDURES/ASSESSMENTS AND SCHEDULE

Planned timepoints for all safety assessments are provided in Table 1.

6.1.1 Clinical Safety Assessments

The following clinical assessments will be performed to assess the safety profile of SPK-8016:

- Physical examination
- Vital signs
- AEs
- Concomitant therapy and procedures.

6.1.2 Laboratory Safety Assessments

The following laboratory tests will be performed to assess the safety profile of SPK-8016

- Hematology
- Clinical chemistry
- Urinalysis
- Liver Function Tests
- Coagulation
- Neutralizing antibody development against FVIII by Bethesda assay (FVIII inhibitor)
- Neutralizing antibody to [redacted]
- Immunology to assess cellular immune responses
- Immune profiling of plasma after vector administration
- Vector shedding (serum, saliva, urine, semen)

6.2 Additional Assessments

6.2.1 Joint Assessments

Baseline clinical status of joints and identification of target joint(s) will be conducted during Screening or pre-vector infusion.

Joint health and identification of target joint(s) will be assessed during this study. A target joint is defined as a major joint (*e.g.,* hip, elbow, wrist, shoulder, knee, and ankle) into which 3 or more spontaneous bleeds occurs in a single joint within a consecutive 6-month period as documented in the medical or home treatment records. Symptoms of pre-existing target joint involvement or hemophilic arthropathy (*e.g.,* synovitis, persistent swelling, effusion, limitation of range of motion) should be documented as part of the Joint assessment.

The Investigator will assess the participant's target joint(s) identified at baseline by location of target joint(s), location of joint bleeding, and frequency of bleeding from each joint.

6.2.2 Hemophilia Joint Health Score

Joint assessment will be conducted using a Hemophilia Joint Health Score (HJHS) (See Section 14.4). HJHS will be performed to evaluate the clinical status of the joints at screening or prior to Day 0, Week 26, and Week 52. This assessment is based on the scoring system used in a joint scoring reliability study in boys with hemophilia (Hilliard, 2006). It has been used as a tool to evaluate musculoskeletal outcomes in a cohort of 20 boys, aged 4 to 17 years (Saulyte Trakymiene, 2010).

Six joints (left ankle [LA], right ankle [RA], left elbow [LE], right elbow [RE], left knee [LK], and right knee [RK]) will be scored according to the following criteria: swelling, muscle atrophy, crepitus, flexion loss, extension loss, instability, joint pain, and strength. Gait will be scored based on walking and climbing stairs.

6.2.3 Activity Assessments (Hemophilia Activities List)

Participants will complete the Hemophilia Activities List (HAL) questionnaire (See Section 14.1) (van Genderen, 2004; van Genderen, 2006). In the questionnaire, several activities are listed that could be difficult for patients with hemophilia.

6.2.4 Participant Questionnaires

The following questionnaires will be administered at selected visits:

- Quality-of-Life (See Section 14.2): The Haem-A-QoL Questionnaire, a hemophilia-specific QoL tool for hemophilia patients who are 17 years old and above, consists of 46 items covering 10 dimensions to assess a patient's health-related quality-of-life (von Mackensen, 2005).
- Health Assessment (See Section 14.3): The EQ-5D-5L Questionnaire is a simple generic measure, containing 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). It is a standardized instrument that was established by the EuroQol Group, providing a simple descriptive profile and a single index value for health status (EuroQol Group, 1990).

6.2.5 Health-economic Assessment

The following information will be captured during the study as part of a health-economic assessment:

a) Number of hospitalizations (excluding pre-planned hospitalizations documented at screening)
b) Number of hospitalization days
c) Number of emergency room visits
d) Number of physician visits, excluding study visits
e) Number of days off from school or work

6.2.6 Archived Bio-samples

At each time-point where samples are collected for FVIII activity, spare plasma will also be collected. Samples will be archived for testing (if required) of further coagulation assays, or for

clarification of any clinical or laboratory AEs. If a participant provides consent, samples may be used for genetic analyses and future research.

6.3 Clinical Procedures

The clinical procedures that will be conducted during this study related to the evaluation of population demographics, medical and hemophilia history, safety, and efficacy are provided in Table 3.

Table 3: Clinical Procedures: Study Assessments

Assessment	Description
Demographics	Date of birth, age, sex, ethnicity, and race
Medical History	Relevant medical history Prior/concomitant therapies within the past 30 days from signing the informed consent
Hemophilia History	Hemophilia A diagnosis and status: • Documented date of and age at diagnosis • FVIII activity level • Blood type and Rh factor • FVIII Genotype and HLA Genotyping • History of inhibitors to FVIII or allergic reactions to FVIII products • Bleed history in the past 52 weeks • Previous therapy in the past 52 weeks
PK profile	• Current FVIII product. o Recovery (maximum drug concentration [Cmax] following a known IU/kg dose of FVIII) o Half-life calculations
Joint Assessments	Target joint assessment in the past 26 weeks: • Location of target joint(s) • Location of joint bleeding • Frequency of bleed per joint • Target joint history o Hemophilic Arthropathy Assessment o HJHS
Physical Exam	• Full physical examination • Height and body weight
Vital Signs	• BP (systolic and diastolic) • Respiratory rate • Pulse rate • Temporal or Oral temperature (oC)
Electrocardiogram	• ECG
Quality of Life questionnaires	Haem-A-QoL EQ-5D-5L
Activity Assessments and Health Economics	Hemophilia Activities List

Assessment	Description
	Health Economic Assessment
Review of AEs and Concomitant Therapies	Review of AEs and concomitant therapies throughout the study
Hematology (CL)	WBC count with Differential ○ Neutrophils ○ Lymphocytes ○ Monocytes ○ Eosinophils ○ Basophils RBC ○ Hemoglobin ○ Hematocrit ○ Platelet count
Clinical Chemistry (CL)	• Sodium • Potassium • Chloride • Bicarbonate • Glucose • Phosphate • Serum creatinine • BUN
Liver Fibrosis Diagnostic Test	• Fibroscan (LL) • FibroTest/Fibrosure (CL) ○ Apolipoprotein A1 ○ Alpha 2 Macroglobulin ○ Haptoglobin • AST-to-Platelet Ratio • Liver Ultrasound • α-fetoprotein
Urinalysis (CL)	• pH • Glucose • Protein • Blood • Ketones
Liver Function Tests and CRP (CL and LL)	• Albumin, • Total bilirubin • Direct bilirubin • Indirect bilirubin • ALP • AST • ALT • Total protein • GGT • LDH • CRP
Coagulation (CL, LL as noted)	• FVIII activity, aPTT (CL and LL) • FVIII antigen (CL) • FVIII inhibitor (Nijmegen Bethesda) (CL, LL at screening), VWF antigen, VWF activity (CL) • Spare Plasma
Neutralizing Antibody (CL)	[redacted] Neutralizing Antibody
Immunology (CL)	• Immun PBMC for ELISpot • Immun PBMC for Inflamm
Immune Profiling (CL)	• Cytokine/Chemokine Analysis • Tryptophan metabolites • Complement activation
PAX Gene (CL)	• PAX Gene (RNA)
Vector Shedding (CL)	• Urine • Serum • Saliva • Semen

Assessment	Description
HBV, HCV (CL)	• Hepatitis B surface antigen • Hepatitis B core antibody total • Hepatitis B core antibody total • HCV RNA Ampliprep Taqman 2.0 • HBV DNA Ampliprep Taqman 2.0 • HBV DNA Ampliprep Taqman 2.0DIL
HIV Serology (CL)	HIV-1/HIV-2 Antibody Screen HIV-1/HIV-2 Antigen Screen
Viral load and CD4 (CL)	HIV-1/HIV-2 Viral Load, CD4+ Count
TPMT (LL)	TPMT activity or genotype
Lipid Profile (LL)	Lipid profile (Cholesterol LDL, VLDL, and HDL, triglycerides)
TB (LL)	Tuberculosis Interferon-gamma Release Assay
SARS-CoV-2 Testing (LL)	anti-SARS-CoV-2 serology NAAT for infection with SARS-CoV-2
Fibrinogen (LL)	Fibrinogen Antigen, D-Dimer, Thrombin Time

AE=adverse event; ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; anti-HBc=total hepatitis B core antibody; aPTT=activated partial thromboplastin time; BP=blood pressure; BUN=blood urea nitrogen; CD4=cluster of differentiation 4; CL=central laboratory; CRP=c-reactive protein; ELISpot=enzyme-linked immunospot assay; EQ-5D-5L=Euro Quality-of-Life Five Dimensions Questionnaire; FVIII=coagulation factor VIII; GGT= gamma-glutamyl transferase; Haem-A-QoL=Hemophilia Quality of Life Questionnaire; HBV-DNA=hepatitis B virus-deoxyribonucleic acid; HBsAg= hepatitis B surface antigen; HCV-Ab=hepatitis C virus antibody; HCV-RNA=hepatitis C virus- ribonucleic acid; HDL=high-density lipoprotein; HIV-1=human immunodeficiency virus 1; HIV-2= human immunodeficiency virus 2; HJHS=hemophilia joint health score; HLA= human leukocyte antigen; LDH= lactate dehydrogenase; LDL=low-density lipoprotein; LL=local laboratory; NAAT=nucleic acid amplification testing; PBMC=peripheral blood mononuclear cells; RBC=red blood cell; Rh factor= rhesus factor; RNA=ribonucleic acid; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; TB=tuberculosis; [redacted]; TPMT=thiopurine methyltransferase; VWF=von Willebrand factor; WBC=white blood cell.

6.4 Screening Period

All participants must provide written informed consent before any study-specific procedures or assessments are performed. 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.

Immunosuppressive therapy may enhance adverse/toxic effects of live vaccines and may diminish the effect of inactivated vaccines. Due to the potential that trial participants may receive an immunosuppressive medication during the SPK-8016-101 trial, it is highly recommended that the Investigator and participant review and complete all age-appropriate vaccinations during the screening period, at least 4 weeks prior to the planned day of administration of SPK-8016.

Anti-SARS-CoV-2 serology testing will be conducted at baseline and at Week 18. This result will be informative only and does not affect trial Inclusion/Exclusion. Multiple effects of COVID-19 on the hemostatic system have been described, including the development of anti-phospholipid antibodies (Lupus-like anticoagulants), which might confound interpretation of one-stage clotting factor VIII assays.

6.4.1 Screening Assessments

The following procedures will be performed during the screening period, which may occur over a period up to a maximum of 16 weeks:

- Obtain written informed consent
- Review inclusion/exclusion criteria
- Review prior and concomitant therapy
- Record information on demographic and baseline characteristics
- Record medical history and hemophilia history, including FVIII treatment history and bleeding history
- Record Target Joints, Hemophilia Joint Health Score, and Joint Health Assessment – *May be done at Screening or prior to Day 0*
- Measure vital signs (blood pressure (systolic and diastolic), pulse, respiratory rate, and oral/temporal temperature)
- Perform physical examination, including height and weight
- Haem-A-QoL, EQ-5D-5L – May be done at Screening or prior to Day 0
- Electrocardiogram (ECG) (*for participants >50 years of age, or if clinically indicated*)
- Obtain samples for central laboratory evaluation, unless otherwise noted:
  - Hematology
  - Clinical Chemistry
  - α-fetoprotein
  - Vector shedding (Serum, saliva, urine, semen) – *May be done at Screening or Day 0 (prior to vector infusion)*
  - Spare Plasma
  - AAV Neutralizing Antibody
  - HBsAg, anti-HBc, HBV-DNA
  - HCV-RNA load assay
  - Urinalysis (using dipstick)
  - Coagulation:
    - aPTT, FVIII Activity, FVIII Inhibitor *(local and central labs)*
    - FVIII Antigen, VWF Activity, VWF antigen
  - Liver function and CRP tests (*local and central lab*)
  - Liver fibrosis diagnostic test, i.e., FibroScan (LL), FibroTest (CL), or AST-to-Platelet Ratio Index (LL)
  - Fibrinogen Antigen (*local lab*)
    - D-Dimer

- Thrombin Time

- Thiopurine methyltransferase (TPMT) (*local lab*)
- TB Interferon-gamma Release Assay (*local lab*)
- Anti-SARS-CoV-2 serology (*local lab*)
- If indicated:
  - HIV1/HIV2 viral load and CD4+ T cell count *(for participants who are HIV-positive),*
    (local lab or central lab),
  - Lipid panel *(for participants with a history of dyslipidemia or hypercholesterolemia, or if clinically indicated)*
  - Liver ultrasound (*"if indicated" means "if, in the judgment of the investigating site or the Sponsor", the liver ultrasound is indicated to aid interpretation of the screening evaluation of liver fibrosis".)*
  - FVIII Genotyping and HLA Genotyping *(Optional sample if genotype is unknown)* *
- Dispense and train on FVIII Infusion Log (*May be done at Screening or Day 0 (prior to vector infusion*)
- Provide PK profile using participants' current FVIII product. This can be obtained from historical values if available and must include recovery (maximum drug concentration [C_{max}] following a known IU/kg dose of FVIII) and half-life calculations. Population PK analysis using 2-4 samples is acceptable. If this is not available on the current FVIII product, the participant must undergo a PK analysis locally – *May be done at Screening or Day 0 (prior to vector infusion)*

*For participants, whose FVIII genotype is unknown, a sample may be drawn for analysis at screening. This is not an inclusion or exclusion criterion; the participant's refusal to have this FVIII genotype sample taken would not exclude the participant from the study unless the severity of the disease is otherwise unable to be verified. FVIII genotyping may provide information regarding the predisposition of genotypic subpopulations to experience different bleeding frequencies and/or to experience different immunologic responses to FVIII following gene therapy.

6.5 Three to Seven Days Prior to Day 0 Assessments

Laboratory assessments should occur within 3 to 7 days prior to Day 0 for Cohorts 1 and 2. Results must be consistent with inclusion/exclusion criteria.

- Liver Function (*local lab*)
- Hematology (*local lab*)
- Immunology (*central lab*)
- Immune Profiling (*central lab*)
- PAX Gene (RNA) (*central lab*)
- SARS-CoV-2 NAAT Testing (*local lab*)

At the discretion of the Investigator, NAAT for infection with SARS-CoV-2 (the infectious agent in COVID-19) may be performed at this time for asymptomatic individuals. NAAT testing should be performed at this time for individuals with any of the following symptoms: cough, shortness of breath or difficulty breathing, fever, chills, sore throat, new loss of taste or smell, new persistent or recurrent muscle pain, and/or headache. Note: The Infectious Disease Society of America panel suggests collecting nasopharyngeal, or mid-turbinate or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing in symptomatic individuals with URTI or ILI suspected of having COVID-19.

The participants in Cohort 1 should be contacted prior to Day -2 to verify no changes in the general health status and confirm the prescribed [redacted] regimen.

6.6 Day -2

[redacted]

6.7 Dosing Day Assessments (Day 0)

If a participant has a bleeding event between the screening period and the start of Day 0, he should be treated with his previous FVIII product. All bleeding events and related treatment should be recorded in the electronic case report form (eCRF).

All participants will administer a prophylactic infusion of 50 IU/kg of their current FVIII concentrate the morning of Day 0. This can be self-administered and recorded in the subject infusion log. Supervision from the site staff is permitted.

[redacted]

All participants will be infused with a single IV infusion of SPK-8016 at the vector-administration center. The complete dose of SPK-8016 will be infused via infusion pump over approximately 60 minutes.

6.7.1 FVIII Dosing

At FVIII dosing:

- Verify the administration (self-infused or assisted by site staff) of a single prophylactic IV infusion of 50 IU/kg of FVIII product on the morning of Day 0.

6.7.2 [redacted]

[redacted]

- [redacted]

6.7.3 Vector Dosing

*Assessments **before** SPK-8016 Vector infusion:*

- Review Inclusion/ Exclusion Criteria
- Measure vital signs (blood pressure, pulse, respiratory rate, and oral/temporal temperature)
- Perform physical examination, including weight
- Hemophilia Activities List, Health Economic Assessment
- Record AEs and concomitant therapies
- Vector shedding, *if not done at screening*
- Immunology
- Immune Profiling
- PAX Gene

*Assessments **during** Vector infusion:*

- Administer a single IV infusion of SPK-8016 via infusion pump over approximately 60 minutes
- Record AEs and concomitant therapies

*Assessments **after completion of** Vector infusion:*

- Vital signs immediately following the infusion (± 2 minutes), and 2 hours (±10 min) after the end of vector infusion
- Obtain blood samples for central laboratory evaluation at 30 (±2) min, 2 hours (±10 min), and 5 hours (±10 min) after the completion of the vector infusion:
  - Immune Profiling
  - PAX Gene (RNA) (at 5 hours [±10 min])
- Record AEs and concomitant therapies

6.7.4 Day 1

Measure vital signs 24 (± 1 hr) hours post-SPK-8016 dose (blood pressure, pulse, respiratory rate, and oral/temporal temperature).

Obtain blood samples for central laboratory evaluation at all visits, unless otherwise noted:

- Immune profiling (CL) (24 (±1) hours post vector infusion)
- PAX Gene (CL) (24 (±1) hours post vector infusion)
- Hematology (CL and LL)
- Coagulation: aPTT and FVIII Activity (CL and LL), FVIII Antigen (CL)
- Liver Function tests and CRP (CL and LL)
- Spare plasma
- Record AEs and concomitant therapies

6.8 Follow-up Observation Period (Weeks 1-52)

Visits during the follow-up period may occur at either the vector-administration or follow-up center. Any visit requiring a physical exam must be performed at the study center. All other visits without a physical exam may be performed by a qualified and trained in-home service provider.

U.S. Centers for Disease Control and Prevention (CDC) guidance on hygiene, travel, and social interactions will be considered by the Investigator and Sponsor to minimize the risk of community acquired viral infections. This may include recommending a shelter in place and/or other social distancing measures during immunomodulation. *FDA Guidance for Industry, Investigators, and Institutional Review Boards on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic* (2020) will provide considerations to assist in assuring the safety of trial participants. International and local health guidelines may be observed for sites outside of the United States.

6.8.1 Days 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59, 63, 66, 70, 73, 77, 84 (± 2 days)

A minimum of twice weekly visits is required from Weeks 1-11 to monitor for a potential rise in hepatic transaminases and/or loss of FVIII transgene expression. Visits at Weeks 1-11 may be performed by an in-home service provider.

If an apparent immune response/liver inflammation is observed and triggers the initiation of reactive corticosteroids, additional samples are to be collected for Immune Profiling and PAX Gene prior to corticosteroid administration, if possible, and then Week 1 (± 2 days) and Week 2 (± 2 days) following initiation of corticosteroids (see Table 1 and Section 4.1.3.1).

If immunomodulation is initiated, weekly visits should continue to collect transaminases, FVIII (local and central lab), Immunology. Weekly hematology and monthly lipid profile results should also be monitored locally for Cohorts 1 and 2 until [redacted] dosing has stopped, and this may be altered based on clinical response. Social distancing may be recommended for the duration of immunomodulation.

The following procedures will be performed at all visits unless otherwise noted:

- Perform physical examination, including weight *(Days 3, 49, 84)*
- Measure vital signs *(Days 3, 49, 84)*
- Hemophilia Activities List, Health Economic Assessment *(Days 49 and 84)*
- Review FVIII Infusion Log *(Days 49, 84)*
- Obtain samples for central laboratory evaluation at all visits, unless otherwise noted:
  - Coagulation
    - FVIII Inhibitor (Days 49, 84)
    - aPTT, FVIII activity *(local and central labs)*
    - FVIII antigen (*central lab*)
  - Liver function tests and CRP *(local and central labs)*
  - Hematology *(Days 3, 49, 84)*
  - Clinical Chemistry *(Days 3, 49, 84)*

  - AAV Neutralizing antibody *(Days 3, 10, 17, 24, 31, 49, 84)*
  - Immunology (*Days 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, 84)*
  - Immune profiling *(Days 7, 14, 21, 28, 56)*
  - PAX Gene (RNA) *(Days 14, 28, 56)*
  - Spare plasma *(Day 84)*
  - Vector shedding samples (serum, saliva, urine, semen)

    NOTE: Vector shedding samples are collected only until the results of 3 consecutive samples are negative and received by the Investigator
- Fibrinogen *(Days 10, 24) (local)*
  - Fibrinogen Antigen
  - D-Dimer
  - Thrombin Time
- Record AEs and concomitant therapies

6.8.2 Weeks 14, 16, 18, 22, 26, 30, 34, 40, 46, 52/End of Study (± 2 weeks)

Visits will occur every 2 weeks from Weeks 12-16 and every 4 weeks from Weeks 18-34, then every 6 weeks from Weeks 40-52/ EOS. If immunomodulation is initiated, weekly visits should continue to collect transaminases, FVIII, and Immunology. Weekly Hematology and monthly lipid profile results should also be monitored regularly in the local lab for Cohorts 1 and 2 until [redacted] dosing has stopped, and this may be altered based on clinical response.

The following procedures will be performed at all visits unless otherwise noted:
- Perform physical examination, including weight *(Weeks 18, 26, 34, 40, 52/EOS)*
- Measure vital signs
- Perform liver ultrasound (*Week 52/EOS, if indicated*)
- Record Target Joints and Joint Health Assessment *(Week 26, 52/EOS)*
- Hemophilia Activities List, Health Economic Assessment *(Weeks 14, 26, 40, 52/EOS)*
- Haem-A-QoL, EQ-5D-5L *(Weeks 26, 52/EOS)*
- Review FVIII Infusion Log *(Weeks 14, 26, 40, 52/EOS)*
- Obtain samples for central laboratory evaluation, unless otherwise noted:
  - Coagulation:
    - aPTT, FVIII activity *(local and central labs)*
    - FVIII antigen
    - FVIII Inhibitor *(Weeks 14, 26, 40, 52)*
    - VWF activity, VWF antigen (*Week 52/EOS only)*
  - Liver function tests and CRP *(local and central labs)*
  - Immunology
  - Immune profiling *(Week 16)*
  - Lipid panel *(Week 52, if indicated)*
  - Vector shedding samples (serum, saliva, urine, semen)

    NOTE: Vector shedding samples are collected only until 3 consecutive samples are negative
  - Hematology *(Weeks 14, 16, 18, 26, 52/EOS)*

  - Clinical Chemistry *(Weeks 18, 26, 52/EOS)*
  - AAV Neutralizing antibody *(Weeks 14, 26, 40, 52/EOS)*
  - Spare plasma *(Weeks 26, 40, 52/EOS)*
  - Urinalysis *(Weeks 26, 52/EOS)*
  - Anti SARS-CoV-2 serology *(Week 18)*
  - α-fetoprotein (*Week 52/EOS*)
- Record AEs and concomitant therapies

If the LTFU study is not enrolling at the time of a participant's planned Week 52/EOS visit, the participant may remain in SPK-8016-101. The Week 52 procedures are to be performed only at the EOS Visit. The procedures from Week 46, except for collection of immunology samples for ELISpot, will be performed at Week 52 and every 12 weeks until the LTFU study is open. At that time, the Week 52/EOS visit should occur.

7 STUDY INTERVENTION

7.1 Description of Study Drug

Study Drug Name:	SPK-8016	
Formulation (including dosage form and strength):	[redacted]	
Route of Administration:	IV infusion	
Manufacturing Process	[redacted]	[redacted]
Packaging and Labeling:	1. SPK-8016 will be provided as a sterile frozen (< -60°C) liquid at a volume of 1.0 mL in a polypropylene sterile 1.5 mL cryogenic screw cap vial. 2. SPK-8016-EC ([redacted]) will be provided as a sterile frozen (< -60°C) liquid at a volume of 1.0 mL in a polypropylene sterile 1.5 mL cryogenic screw cap vial. 3. Study drug will be labeled as required per country requirements.	1. SPK-8016 will be provided as a sterile frozen (≤ -65°C) liquid at a volume of 1.0 mL in a 2 mL Crystal Zenith® vial with stopper and flip-off seal. 2. Study drug will be labeled as required per country requirements.

Immunomodulators may be provided by the Sponsor. Refer to the most recent version of the Investigator's and Pharmacy Brochures for further details on the study drug and immunomodulators.

7.2 Study Drug Doses

The proposed SPK-8016 doses for this study are:

- Starting dose ($5x10^{11}$ vg/kg)
- Middle dose ($1x10^{12}$ vg/kg)
- High dose ($2x10^{12}$ vg/kg)

Any decision to add a fourth dose level or further expand one of the existing protocol-defined dose levels will be made in consultation with the DMC.

Once at least 2 participants of a given dose level complete 6 weeks of safety evaluation and the safety data have been reviewed by the DMC without any safety concern, then the first participant at the next dose level can be infused with SPK-8016. Dosage for each participant will be

calculated according to the dose calculation worksheet and verified by the Sponsor. Weight obtained at Screening may be used for dose calculations.

For participants with body mass index (BMI) exceeding 30 kg/m^2, the study dose will be calculated based on an alternative body weight determination that assumes a maximum permissible BMI of 30 kg/m^2. For example, a participant who is 6'2" and weighs 370 pounds (BMI 47.5 kg/m^2) would receive a vector dose based on an alternative body weight of 234 pounds (which is the body weight associated with a BMI of 30 kg/m^2 for a 6'2" individual).

7.3 Dose Schedule and Administration

[redacted]

7.4 Treatment Compliance

Compliance with the infusion of FVIII protein product and SPK-8016 on the morning of Day 0 will be verified and recorded by trained site staff.

Participants will self-infuse with 50 IU/kg of their usual FVIII protein product per the product insert. Supervision from the site staff is permitted. Number of vials, total volume, and total dosage will be monitored and recorded by the site staff.

Cohort 1 will initiate [redacted] approximately 48 hours prior to Day 0. The time of administration will be monitored and recorded by the study staff. Participants in Cohort 2 will be infused with [redacted] after FVIII administration and will be supervised by the study staff. The relevant information will be monitored and recorded by the site staff.

[redacted]

7.5 Study Drug Storage

SPK-8016 must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions, as outlined in the Pharmacy Brochure, with access limited to the Investigator and authorized site staff. Sponsor-provided immunomodulators must be stored in a controlled environment under conditions outlined in the Pharmacy Brochure.

7.6 Study Drug Preparation, Handling and Disposal

7.6.1 Study Drug Preparation

SPK-8016 is derived from a virus and should be considered and handled as an infectious agent.

A qualified pharmacist with specific training on this protocol will be responsible for vector receipt from the Sponsor, storage, documentation of traceability of investigational product at the investigational site, and preparation on the day of administration.

Prior to preparing the study drug and immunomodulators, the responsible pharmacy staff should first carefully review the instructions provided in the Pharmacy Brochure and/or relevant product labeling. The investigational pharmacy personnel preparing the study drug for infusion will use universal precautions and appropriate personal protective equipment. The study drug dilution will be performed using aseptic techniques in a Class II biosafety cabinet. Just prior to use, the frozen study drug product will be thawed at room temperature and diluted according to the verified dose calculation worksheet. The diluted SPK-8016 product (i.e., for IV infusion), will be stored at room temperature and infusion must be initiated within 6 hours of thawing to assure maximum potency.

7.6.2 Study Drug Handling and Disposal

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit and on-site storage for all study drug and immunomodulators received, and any discrepancies are reported and resolved before use of the study drug or immunomodulator. Immediately after receipt, the study drug and immunomodulators should be stored as described in Section 7.5. Refer to the Pharmacy Brochure for management and reporting of any out-of-range temperatures.

Only participants enrolled in the study may receive study drug or immunomodulator and only trained and authorized site staff may prepare, dispense and/or administer (except self-administered medications) the study drug and immunomodulators. Study drug must only be thawed after confirmation that the participant is eligible, if applicable. Vials are for single use only.

The site should store all used and unused vials of SPK-8016 and immunomodulators, as instructed by the Sponsor and as per the institution's procedures.

7.6.3 Accountability and Destruction

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposal records). Accountability must be maintained for SPK-8016 and immunomodulators.

Participant returns of self-administered (take home) immunomodulators provided by the Sponsor must be documented by site personnel and retained at the site until reconciliation, where allowable per institutional policy, by the study monitor. At study completion, reconciliation must be made between the amount of study drug and immunomodulators supplied, dispensed, and subsequently destroyed or returned to the Sponsor, or its designee.

Further guidance and information for the final disposal of used/unused study drug and Sponsor-provided immunomodulators are provided in the Pharmacy Brochure.

7.7 Labeling

The SPK-8016 product label includes investigational product name; manufacturer; specific lot number; date of manufacture; vial number; storage instructions; and investigational product warning. The immunomodulator product labels include similar information. The Pharmacy Brochure will contain copies of all approved labels.

7.8 Study Compliance

Following the administration of SPK-8016, participants will be followed according to the protocol schedule of assessments. Participants will be encouraged to follow-up completely and according to study endpoints. Non-adherence to the protocol will be reported to the relevant regulatory groups overseeing the study.

7.9 Prior and Concomitant Medications

The use of concomitant therapies or procedures, as defined below, must be recorded on the participant's eCRF. AEs related to administration of these therapies or procedures must be documented on the appropriate eCRF.

7.9.1 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving 30 days prior to screening, at the time of enrollment or during the study must be recorded in the eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

Participants taking medication routinely for a pre-existing condition should be on a regimen, which has been stable for at least 3 weeks, and dosage changes should not be anticipated during the post-infusion period for this study. Any dose change during the study must be documented in the participant's record and eCRF. The Investigator or designee should review the list of all current medications, over the counter medications, vitamins, and herbal supplements. Any concomitant medication that has known hepatoxicity should be discontinued in the first 12 weeks following vector infusion. The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

7.9.2 Permitted Therapy

During the study, participants are requested to suspend their prophylaxis regimen, but participants are permitted to take:

- FVIII product, as needed for bleeding. Usage of clotting factors (product, date, dosage, reason) will be recorded in the infusion log. The use of FVIII for prophylaxis is allowed

during the first 14 days post SPK-8016 infusion if deemed medically necessary by the Principal Investigator.
- Non-steroidal anti-inflammatory drugs, except ibuprofen and acetylsalicylic acid (aspirin).

Participants should be instructed to discuss any new medications, including non-prescription drugs and herbal preparations, with the Investigator prior to taking them.

Other therapies considered necessary for the participant's welfare may be given at the discretion of the Investigator. All such therapies must be recorded in the eCRF.

The CDC recommends a limit of 2 alcoholic drinks per day or fewer for adult males. While this is a general guideline, the Sponsor recommends not having any alcohol drinks during the first 12 weeks after infusion of the study product. Alcohol has the potential to inflame the liver, which may make it difficult to monitor the status of liver health and has the potential to interact negatively with the study product or other medications.

7.9.3 Prohibited Therapy

The following concomitant medications are not permitted during the study:

- Acetylsalicylic acid (aspirin) or ibuprofen; however, other non-steroidal anti-inflammatory drugs are permitted.
- Routine prophylactic treatment with FVIII product after Day 14 post vector infusion unless clinically indicated and in discussion with the Investigator and/or Sponsor.
- Routine prophylactic treatment with emicizumab unless clinically indicated and in discussion with the Investigator and/or Sponsor
- Any other investigational therapies prior to screening as defined in the exclusion criteria or used during the study

Any medication or herbal supplement that may cause liver toxicity should be avoided. If a medication with known liver toxicity cannot be avoided, the Investigator should discuss it with the Sponsor.

7.9.4 Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy, tooth extraction) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the time the participant is enrolled (at screening) and the last study visit (Week 52/EOS). The use of concomitant procedures must be recorded in the eCRF.

8 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

8.1 Participant Discontinuation/Withdrawal from the Study

Participants may withdraw from the study at any time at their own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study and also withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such withdrawal of consent.

The Investigator should record the reason and date of withdrawal in the eCRF and in the participant's medical records. If possible, the participant should confirm his decision in writing.

Participants who withdraw from the study after being dosed should return for a final visit within the timeframe of their next scheduled visit and complete all EOS assessments (see Table 1).

A participant may permanently withdraw from the study at any time. An investigator and the Sponsor may permanently remove a participant from the study at their discretion for any of the following reasons:

- The participant withdraws consent.
- At the discretion of the Investigator for medical reasons.
- At the discretion of the Investigator or Sponsor for non-compliance.
- The participant is unwilling or unable to attend study visits and undergo safety assessments as per the protocol.

If a participant withdraws from the study after enrollment, but before receiving a dose of the study drug (SPK-8016), then follow-up beyond the screening evaluations is not required. Withdrawn participants that did not receive a dose of the study product will be replaced.

8.2 Early Termination Study Visit

If the participant withdraws after receiving the investigational product, the early termination study procedures should be identical to those of the EOS visit. If the participant withdraws before receiving the investigational product, early termination study procedures are not necessary.

8.3 Lost to Follow Up

A participant will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and there is documentation that he/she has been 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 document the attempt to contact the participant and reschedule the missed visit as soon as possible. The site should counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if 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. If the participant provided a telephone contact number, 3 telephone call attempts will be made to the participant's last known phone number. In addition, a certified letter will be sent to the participant's last known mailing address (or local equivalent methods). These contact attempts will be documented in the participant's study record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

9 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The definitions of an AE or SAE can be found in the following sections.

An AE 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 for pursuing follow-up information for AEs or SAEs (see Section 9.4.1).

9.1 Definitions

9.1.1 Adverse Event

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

All AEs must be documented regardless of causality.

Events meeting the AE definition include:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present at one time before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product 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/self-harming intent. Such overdoses should be reported regardless of sequelae.

Lack of perceived efficacy, failure of expected pharmacological action, or similar assessment will not necessarily be reported as an AE. Such instances will be captured in the efficacy assessments. However, if signs, symptoms and/or clinical sequelae resulting from lack of efficacy meet the definition of an SAE, they will be reported as such.

Events that do NOT meet the AE definition include:

- In this population, bleeding episodes are not considered AEs. All bleeding episodes will be captured in the eCRF. If serious criteria apply (see Section 9.1.2), the event should be reported as an SAE.

- 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 procedures (e.g., endoscopy, appendectomy) should not be reported as AEs, but should be reported as the condition that led to the procedure as 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.

9.1.2 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 (e.g., 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 meets the following criteria:

- **Results in death**
- **Is life-threatening**

  The term 'life-threatening' in the definition of 'serious' refers to an event 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.

- **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 department 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.

- **Results in persistent or significant 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 (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

  - Is a congenital anomaly/birth defect
  - Other Medically Important conditions:

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.

A prescheduled, elective procedure, or a routinely scheduled treatment that requires hospitalization is NOT considered as to be an SAE; the study site must document all the following:

- The prescheduled, or elective procedure, or routinely scheduled treatment was scheduled (or participant was on a waiting list to be scheduled) prior to obtaining the participant's consent to participate in the study.
- The condition requiring the prescheduled, or elective procedure, or routinely scheduled treatment was present before and did not worsen or progress, in the opinion of the Investigator, between the participant's consent to participate in the study and the time of the procedure or treatment. The prescheduled, or elective procedure, or routinely scheduled treatment is the sole reason for the intervention or hospital admission.

9.1.3 Adverse Events of Special Interest (AESI)

There are several AEs that will be monitored closely during the study as adverse events of special interest (AESIs) to enable an adequate risk-benefit evaluation of SPK-8016 versus standard therapy during the study and additional data may be requested for these events. The AESIs are:

- Any AEs associated with FVIII inhibitor formation
- The development, in any participant, of SPK-8016 related (>10x ULN) elevated transaminases
- The development, in any participant, of SPK-8016 related (≥2.5x ULN) elevated hepatic transaminases that fails to resolve to less than 2.5x ULN within 4 weeks with immunomodulatory agents
- Any occurrence of a malignancy at any point after vector infusion that is related to SPK-8016 or one of the immune modulating agents
- Any thrombotic and/or embolic events (TEE)

9.2 Recording of AE and/or SAE

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.

- The Investigator will then record all relevant AE/SAE information in the eCRF.
- It is not acceptable for the Investigator to send photocopies of the participant's medical records to Sponsor/designee in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by Sponsor/designee. In this case, all participant identifiers, with the exception of the participant number, will be redacted on all copies of the medical records before submission to Sponsor/designee.

9.3 Safety Classifications

9.3.1 Assessment of Intensity

The Investigator will assess the intensity for each AE and SAE reported during the study and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least 1 of the predefined criteria as described in the definition of an SAE (see Section 9.1.2), NOT when it is assessed as having an intensity of severe.

Other measures to evaluate AEs and SAEs (especially for abnormal laboratory changes) may be utilized (e.g., National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE v5.0]).

9.3.2 Assessment of Causality

The Investigator is obligated to assess the relationship between investigational product (or administration procedure or study required concomitant therapy) and occurrence of each AE/SAE.

The causality assessment is one of the criteria used when determining regulatory reporting requirements. The causality assessment will be categorized either as:

- Related – there are facts (evidence) or arguments to suggest a "reasonable possibility" for a causal relationship between the investigational product (or administration procedure or study required concomitant therapy) and the event.
- Not Related – there is no reasonable temporal sequence or known pattern of response after administration of the investigational product and/or the AE could have been produced by the participant's clinical state, environmental or toxic factors, or other therapy administered to the participant.

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 investigational product administration will be considered and investigated.

The Investigator will also consult the Investigator's Brochure (IB) 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 Spark or designee. However, it is very important that the Investigator always assess the causality for every event before the initial transmission of the SAE data to Spark or designee.

9.4 Follow-up and Reporting Requirements

9.4.1 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. All AE/SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

New or updated information will be recorded in the originally completed eCRF and SAE form.

The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

9.4.2 Reporting of SAEs

Reporting to the Sponsor/designee via a SAE form will be performed as follows:

- All SAEs must be recorded in the eCRF and reported in the SAE form to the Sponsor within 24 hours of site awareness via email to Spark@primevigilance.com.
- In rare circumstances where SAE information is discussed during a phone conversation with the medical monitor, this SAE information must be documented and immediately sent via email to Spark PV at sparkpv@sparktx.com. A copy of the SAE form must be submitted following the telephone conversation to the SAE reporting email address.
- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE form pages within the designated reporting time frames.
- Contacts for SAE reporting can be found on the SAE form

The minimum reporting requirements for immediate reporting of SAEs include:

- Participant ID
- Event description
- Identifiable reporting source
- Investigator causality assessment

9.5 Time Period and Frequency for Collecting AE and/or SAE Information

9.5.1 Time Period and Frequency for Collecting of AEs and SAEs

All AEs and SAEs will be recorded during the period from the signing of the informed consent form (ICF) until the EOS visit at the time points specified in the Schedule of Events.

Medical occurrences that begin before signing of the informed consent form will be recorded in the Medical History section of the eCRF not in the AE section.

9.5.2 Collection of AEs and SAEs information after conclusion of the study

Investigators are not obligated to actively seek AE or SAE information after conclusion of the 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 study intervention or study participation, the Investigator must promptly notify the Sponsor.

The method of recording, evaluating, and assessing causality of SAEs and the procedures for completing and transmitting SAE reports are provided in Section 9.2, Section 9.3, and Section 9.4.

9.5.3 Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of a 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, IRB/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An Investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.5.4 Pregnancy

Participants included in this study are exclusively male. Should a female partner of the participant become pregnant during the study, the Investigator must notify Spark within 14 days of the Investigator becoming aware of the pregnancy. The participant and female partner must sign an IRB approved pregnant partner release of medical information/ICF before the Investigator can collect medical information about the pregnancy.

Whenever possible, and after a pregnant partner's release of medical information/ICF is signed, a pregnancy in a female partner of a male participant exposed to SPK-8016 should be followed to term so as to assess any potential occurrence of congenital anomalies or birth defects. Any follow-up information, including premature termination and the status of the mother and child after delivery, should be reported by the Investigator to Spark using a Pregnancy Reporting/Outcome Form.

9.6 Treatment of Overdose

The chance of overdosing is remote as the SPK-8016 gene therapy product is a one-time administration with no participant access to the study drug. Furthermore, SPK-8016 will be prepared by trained pharmacy staff and verified by a trained pharmacist before administration to the participant. Nevertheless, for this study, an overdose is any dose given to a participant that is 2 X greater than the intended dose of the study drug. If an adverse event(s) is associated with ("results from") the overdose, the adverse event(s) and overdose will be reported as serious adverse events, even if no other seriousness criteria are met. If the overdose did not result in any associated clinical symptoms or abnormal laboratory results, the overdose will be reported as a non-serious adverse event as "accidental overdose without adverse effect". All overdoses with and without an adverse event should be recorded in the eCRF and only overdose with associated AEs should be reported in an SAE form and sent to the Sponsor/designee within 24 hours. The participant should be monitored and should be treated as medically indicated based on their condition.

In the event of an overdose, the Investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for any AE/SAE and LFT abnormalities. LFTs should be monitored as per protocol and any abnormalities should be immediately reported to the Medical Monitor.
3. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Hypotheses

No statistical hypotheses will be tested as part of this protocol. This study will be used to establish an initial safety and efficacy profile of SPK-8016.

10.2 Sample Size Determination

The sample size is based on the need to establish the initial safety and efficacy profile of SPK-8016. Up to 40 eligible participants will be dosed. If more than 40 eligible participants are to be dosed, regulator(s) will be informed along with DMC recommendation.

Because the size of the hemophilia A population is limited (an estimated incidence of 1 in 5,000 male births), the number of participants available for study is correspondingly limited. Thus, the sample size is based on clinical, rather than statistical, considerations.

10.3 Populations for Analyses

The Full Analysis Set (FAS) is defined as all participants who receive the infusion of SPK-8016. The analyses of efficacy will be performed in this population, including the evaluation of vector-derived FVIII:C activity for estimation of peak and steady-state activity levels. As this is an open-label study, additional subgroup analyses may be specified following clinical review.

The Safety Population will include all participants who receive any study-mandated medication and will be used to summarize all safety data.

10.4 Demography and Baseline Disease Characteristics

All participants who receive the infusion of SPK-8016 will be included in the analysis of demography and baseline disease characteristics.

Demographic and other baseline characteristics will be summarized using descriptive statistics. Data to be tabulated will include, but not be limited to age, race, medical/hemophilia history, and other disease-specific measures. All data will be summarized by the overall study population and by dose level.

10.5 Primary and Secondary Endpoints

10.5.1 Safety Analysis

For the analysis of safety, the incidence and severity of AEs will be tabulated. Changes from baseline in clinical laboratory variables (including FVIII inhibitor and laboratory parameters for thrombotic potential), vital signs, and physical examination findings will be summarized by descriptive statistics.

All adverse events will be tabulated by occurrence, grouped by body system, and summarized by dose group. The rate of occurrence for each adverse event will be summarized by body system, severity, and relation to the administration of SPK-8016. All SAEs will be summarized separately.

Physical examination and clinical laboratory value abnormalities will be summarized by time point. Values and changes from baseline of key safety parameters (e.g., LFTs) at each time point will be tabulated. Exploratory analysis of safety parameters may be done by tabulating transitions from baseline to the EOS visit at Week 52 (e.g., normal to normal, normal to abnormal, etc.).

Descriptive statistics will be used to evaluate the occurrence of hepatic transaminase elevation requiring immunosuppression and the incidence of immune responses to AAV capsid protein and [redacted].

10.5.2 Efficacy Analysis

Summary statistics will be created by SPK-8016 dose group for the following parameters:

- Peak and steady-state FVIII activity levels assessed by coagulation clotting assays. In this study, steady-state levels are based on FVIII:C measurements starting 12 weeks post vector administration and without the use of exogenous FVIII products since vector administration. See Section 10.5.3 for additional details.
- The number of bleeding events expressed as the ABR per participant (spontaneous and traumatic combined) following SPK-8016 administration and beginning 28 days following SPK-8016 administration.
- Absolute change in ABR for all bleeding events and for bleeding events observed beginning 28 days following SPK-8016 administration from the hemophilia history-based ABR for the 52 weeks prior to SPK-8016 administration.
- Proportion of participants with a post SPK-8016 administration ABR of 0 or 1 (separately for all bleeding events and for bleeding events reported beginning at least 28 days following SPK-8016 administration).
- Number of recorded FVIII infusions per participant (referred to in the protocol as annualized FVIII usage) following SPK-8016 administration and beginning 28 days following SPK-8016 administration.
- Absolute change in the number of recorded FVIII infusions following SPK-8016 administration (all infusions and infusions reported beginning 28 days following SPK-8016 administration) from the prior 52-week hemophilia history.

10.5.3 Pharmacokinetics Analysis

FVIII:C activity level (one stage; central lab-recorded) at nominal weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 following SPK-8016 administration will be summarized descriptively by SPK-8016 dose group. For each participant, the difference between the lab visit date and the SPK-8016 infusion date will be obtained as the number of days of follow-up. The nominal week of follow-up will then be computed as the ceiling value of the number of days of follow-up divided by 7. Thus, as an example, nominal week 4 will be assigned to days 22 through 28 of follow-up. An average value will then be computed for all recorded data for the 4 nominal weeks up to a specific time point as defined above, e.g., the nominal week 16 activity level will be the average for all values obtained across nominal weeks 13, 14, 15 and 16. In the event of missing information, the available non-missing data will be used in this calculation, e.g., a participant

with no calculated nominal week 14 FVIII:C value would have the available nominal week 13, 15 and 16 data used to estimate the nominal week 16 result.

Steady-state FVIII:C activity (central lab-recorded) from nominal week 12 through nominal week 52 following SPK-8016 administration will be summarized descriptively by SPK-8016 dose group. Using the same 4-week block construct already identified, an average value for each participant across all time points beginning with nominal week 12 will be computed, i.e., the average FVIII:C level across the individual participant's averages obtained across nominal weeks 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52. The proportion of participants who maintain a FVIII:C activity level ≥ 12% across nominal weeks 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 will also be obtained. Depending on the observed distribution, additional exploratory calculations may be performed to evaluate the proportion of participants with a FVIII:C activity level ≥ 12% across, for example, at least 9 of these 11 timepoints to account for expected random variability. It is expected that corticosteroid therapy will increase the transgene expression from the SPK-8016 expression cassette; this effect is transient and dose-dependent so that as the corticosteroid is tapered and discontinued, the steady state FVIII expression from transduced hepatocytes becomes evident (not influenced by concurrent steroids). The assay of the FVIII activity that is measured while on corticosteroids (as assayed by one-stage and by chromogenic FVIII activity) appears not to be confounded, i.e., while on corticosteroids the observed hemostatic protection afforded by the transgene-derived FVIII is consistent with the measured FVIII activity and the increased measured level of FVIII is not an assay artifact. In contrast, [redacted] is expected not to influence levels of expression of the transgene product, as evaluated in pre-clinical animal studies (summarized in Section 2.2.4) and is expected not to have ongoing activity in circulation after week 12 (the timepoint after which the steady state FVIII:C activity is examined). For this reason, the treatment effect, i.e., determination of the ultimate steady state of FVIII:C activity resulting from the SPK-8016 dose, is not estimated while participants remain on corticosteroid therapy, and is evaluated after corticosteroids have been discontinued completely for at least 2-4 weeks. Additionally, the evaluation of the peak vector-derived FVIII:C activity may require careful interpretation if the participant is receiving corticosteroids at the time of peak activity, whereas [redacted] is not expected to have direct action on transgene expression and peak FVIII:C activity. One potential outcome at the conclusion of the study is that the peak FVIII:C activity may demonstrate a positive association with eventual steady state FVIII:C activity for participants who are not receiving reactive corticosteroids at time of peak activity (in Cohort 1 and Cohort 2) but may fail to demonstrate an association for participants whose peak FVIII:C activity occurs during concurrent treatment with corticosteroids. This potential confounding effect will be considered in the descriptive statistical review.

Vector-shedding of SPK-8016 or time to Below Quantifiable Limits (BQL) will be summarized descriptively by dose group and by PBMC and bodily fluids.

10.6 Exploratory Endpoints Analysis

All data on the new target joints, joint health score, changes in level of activity, hemophilia activities list, and Haem-A-QoL and EQ-5D-5L questionnaires, as described in Section 6.2, will be summarized descriptively by SPK-8016 dose group and provided in participant listings. The questionnaires will be scored according to the recommendations of the questionnaire authors.

Each domain score and change from baseline in domain scores will be summarized by visit. Full methods of analysis for this data will be presented in the Statistical Analysis Plan (SAP).

All data on the joints and health-economic parameters will be summarized for exploratory purposes only. Data will be analyzed according to related recommendations and guidelines. Continuous variables will be summarized by descriptive statistics and categorical variables will be presented with the number and percentage in each category. No imputation of data will be performed.

Exploratory inflammatory profiling of plasma and immune function gene expression of PBMC after vector administration (ELISpot, and other exploratory biomarkers) are described in Section 3 and will be summarized descriptively by SPK-8016 dose group.

10.7 Interim Analyses

Interim analyses – may be performed after at least 2 participants from a given dose cohort complete Week 12.

The SAP will describe the planned interim analyses in greater detail.

10.8 End of Study Definition

A participant is considered to have completed the study if he has completed all phases of the study including the EOS visit in Table 1.

The EOS is defined as the date of the last participant's last visit (LPLV).

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory, Ethical and Study Oversight Considerations

The Sponsor and the Investigator(s) will comply with all instructions, regulations, and agreements in this protocol, and with applicable ICH GCP guidelines, and will conduct the study according to applicable local regulations. The Sponsor and the Investigator(s) must adhere to the principles set forth by the Declaration of Helsinki dated October 2008.

11.1.1 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 GCP Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (e.g., advertisements) must be 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.

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

11.1.2 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 course of the study and for 1 year after completion of the study.

11.1.3 Informed Consent Process

- The Investigator or his/her designee will explain the nature of the study to the participant or his legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA), where applicable, and the IRB/IEC or study center.
- 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 ICF.
- Participants must be re-consented to the most current version of the ICF during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Pregnant partners of male participants will be asked to sign a separate pregnant partner release of medical information/ICF to obtain pregnancy outcomes.

11.1.4 Data Protection

Prior to any testing under this protocol, including screening tests and assessments, candidates must also provide all authorizations required by local law (e.g., HIPAA authorization in North America).

The participant will not be identified by name in the eCRF or in any study reports and these reports will be used for research purposes only. Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will be redacted.

The participant must be informed that his medical records may be examined by Clinical Quality Assurance (CQA) auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities. Every effort will be made to keep the participant's personal medical data confidential.

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

11.1.5 Committee Structure

The independent DMC is composed of at least 3 independent experts in hemophilia or immunology. The independent DMC will be responsible for reviewing safety and efficacy periodically over the course of the study. The specifics regarding the DMC organization and procedures will be outlined in the DMC Charter.

11.1.6 Dissemination of Clinical Study Data

The Sponsor will register the study and post study results, regardless of outcome, on a publicly accessible website (e.g., www.ClinicalTrials.gov), in accordance with the applicable laws and regulations. The Sponsor may also provide study information for inclusion in national registries according to local regulatory requirements. Results of this study will be disclosed according to the relevant regulatory requirements.

11.1.7 Data Quality Assurance

- All participant data relating to the study will be recorded using an eCRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- 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 eCRF 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 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; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and the FDA is notified; or, for a longer retention period if local regulations or institutional policies so require. 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.

11.1.8 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's site.

Data 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.

11.1.9 Study and Site Closure

Study completion is defined as the date that the final participant was examined or received an intervention for the purposes of final collection of data for the primary outcome. A study site is considered closed when all eCRFs have been signed by the Investigator and locked, all required documents and study supplies have been collected and a study-site closure visit has been performed. The Sponsor 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.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

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

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the Investigator
- Discontinuation of further study development

11.1.10 Publication Policy

All study data and intellectual property rights in the results derived from the study are the property of the Sponsor. The Sponsor may utilize the data in various ways, such as submission to government regulatory authorities or disclosure to other Investigators. The Sponsor recognizes that the rights of the Investigator, although he/she is free to utilize data derived from the study for scientific purposes or publish the results in recognized scientific journals, are subject to the provisions of the Clinical Trial Agreement (CTA) upon completion of the study.

Unless otherwise specified in the CTA, the Investigator's institution and Investigator(s) shall not publish or present data from an individual study center until after publication of the results of the complete multicenter study. Subsequent publications must refer to the multicenter findings. The institution and Investigator shall submit reports, abstracts, manuscripts, and/or other presentation materials to the Sponsor for review and approval before submission for publication or presentation. The Sponsor shall have 30 days in advance of submission to respond with any requested revisions, including, without limitation, the deletion of confidential information. The Investigator(s) shall act in good faith upon requested revisions and shall delete any confidential information from such proposed publication.

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13 APPENDIX 1: FACTOR VIII INFUSION LOG

Factor VIII Infusion Log *(Review at all visits)* Subject ID ______

Date and Time of FVIII Injection	Date and Time of Bleed	Name of FVIII Product	FVIII Product Dose (IU)	Reason for Infusion	For a Bleed, Enter Location (Include Left or Right, if applicable)	Bleed Type	Study Staff Initials and Date of Completion
				☐ Prophylaxis ☐ 1st spontaneous bleed txn ☐ F/u spontaneous bleed txn ☐ 1st traumatic bleed txn ☐ F/u traumatic bleed txn ☐ Surgery ☐ Other		☐ Joint ☐ Soft tissue, muscle ☐ Soft tissue, other ☐ Within a body cavity ☐ Intracranial ☐ Surgical Site	
				☐ Prophylaxis ☐ 1st spontaneous bleed txn ☐ F/u spontaneous bleed txn ☐ 1st traumatic bleed txn ☐ F/u traumatic bleed txn ☐ Surgery ☐ Other		☐ Joint ☐ Soft tissue, muscle ☐ Soft tissue, other ☐ Within a body cavity ☐ Intracranial ☐ Surgical Site	
				☐ Prophylaxis ☐ 1st spontaneous bleed txn ☐ F/u spontaneous bleed txn ☐ 1st traumatic bleed txn ☐ F/u traumatic bleed txn ☐ Surgery ☐ Other		☐ Joint ☐ Soft tissue, muscle ☐ Soft tissue, other ☐ Within a body cavity ☐ Intracranial ☐ Surgical Site	
				☐ Prophylaxis ☐ 1st spontaneous bleed txn ☐ F/u spontaneous bleed txn ☐ 1st traumatic bleed txn ☐ F/u traumatic bleed txn ☐ Surgery ☐ Other		☐ Joint ☐ Soft tissue, muscle ☐ Soft tissue, other ☐ Within a body cavity ☐ Intracranial ☐ Surgical Site	

SD Version Date: 25-JAN-2017

____
Signature and Date
(person who completed the form)

14 APPENDIX 2: HEMOPHILIA ASSESSMENTS

14.1 HEMOPHILIA ACTIVITIES LIST

UMC Utrecht
Van Creveldkliniek

Hemophilia Activities List

Date : .....

Patient ID: .....

Version 2.0 2015
USA / Canadian Version

When using this questionnaire, please use the following references:

Van Genderen FR, Van Meeteren NLU, Van der Bom JG, Heijnen L, De Kleijn P, Van den Berg HM, Helders PJM. Functional consequences of haemophilia in adults: the development of the Haemophilia Activities List. Haemophilia *2004;* ***10****: 565-71.*

Van Genderen FR, Westers P, Heijnen L, De Kleijn P, Van den Berg HM, Helders PJM, Van Meeteren NLU. Measuring patients' perceptions on their functional abilities: validation of the Haemophilia Activities List (HAL). Haemophilia *2006;* ***12****: 36-46.*

Introduction

This is the Hemophilia Activities List, or HAL. In this questionnaire several activities are listed that could be difficult for adults who have hemophilia. The aim of this questionnaire is to see how easy it is for you to do these activities.

General comments

When answering the questions, it is only **your own** experience that counts. For every activity, you are asked whether you had any difficulty in performing that activity **due to hemophilia**. There are seven different response options. Answer each question by placing an "X" in the box that describes your situation.

Example:

In the past month, did you have any difficulty **due to hemophilia** with:

	n/a	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Using public transportation (bus, train, subway, streetcar)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Please choose only one box per question. The "N/A" response option ("not applicable") can be used if you never (had to) perform that specific activity. The "N/A" option is only available for some activities. The difference between the "Impossible" and "Always" response option, is that with "Always" you were in fact able to perform that activity, but with problems and with "Impossible" you are unable to perform that activity.

It is very important that you answer all questions. Even when a question seems irrelevant to you, or when you have no opinion relating to the question, please mark the box that describes your situation most closely.

It will take 5-10 minutes to finish this questionnaire.

Lying down / sitting / kneeling / standing

In the previous month, did you have any difficulty, due to hemophilia, with:

	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Sitting down (e.g. on a chair or couch)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Standing up from a chair *that has* armrests	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Standing up from a chair *that does not have* armrests	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Kneeling / squatting	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Bending forward	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Kneeling for long periods of time	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Squatting for long periods of time	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Standing for long periods of time	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Hemophilia Activities List

UMC Utrecht Van Creveldkliniek

Legs

In the previous month, did you have any difficulty, due to hemophilia, with:

	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Walking short distances (less than 0.6 miles / less than 15 minutes)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Walking long distances (more than 0.6 miles / more than 15 minutes)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Walking on a soft surface (e.g. on the beach)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Walking on an uneven surface (e.g. cobblestones, high sidewalks)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Strolling / (window-)shopping	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Walking *up* a flight of stairs (a flight of stairs is approximately 14 steps)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Climbing *down* a flight of stairs	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Running (e.g. in order to catch the bus)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Jumping	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Hemophilia Activities List

UMC Utrecht Van Creveldkliniek

Arms

In the previous month, did you have any difficulty, due to hemophilia, with:

	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Lifting heavy objects	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Carrying heavy objects in the arms	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Fine hand movements (e.g. doing up buttons)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Reaching above your head (to pick something up from a high shelf)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Use of transportation

In the previous month, did you have any difficulty due to hemophilia, with:

	n/a	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Riding a bicycle	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Getting in and out of a car	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Using public transportation (bus, train, subway)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Hemophilia Activities List

UMC Utrecht Van Creveldkliniek

Self care

In the previous month, did you have any difficulty due to hemophilia, with:

	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Drying your whole body	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Putting on a shirt, sweater etc.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Putting on socks and shoes	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Putting on a tie or closing the top button of a shirt	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Going to the toilet	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

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Household tasks

In the previous month, did you have any difficulty, due to hemophilia, with:

	n/a	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Going out shopping (for food, drink etc.)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Washing the dishes, cleaning the sink	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Cleaning the house	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Other household tasks (ironing, making the beds)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Doing odd jobs (both in and around the house)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Gardening	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Leisure activities and sports

In the previous month, did you have any difficulty due to hemophilia with:

	n/a	Impossible	Always a problem	Mostly a problem	Sometimes a problem	Rarely a problem	Never a problem
Playing games (outdoors, e.g. with your children)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Sports	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Going out (theatre / museum / movie theatre / bar)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Hobbies	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Dancing	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Going on a vacation (active)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Going on a vacation ("passive"; beach-/hotel holiday)	☐ 8	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Adaptations and using an aid

To do some activities, you might need some adaptations or an aid. We want to know about the aids that you used on a typical day (so do not include the use of crutches after a joint bleed). The questions below ask about your adaptations or aids.

Do you own a car with adaptations?

- ☐ No, I don't have a car
- ☐ No, I don't have adaptations in my car

Yes, I own a car with (multiple responses are allowed):

- ☐ Electronic windows
- ☐ Power steering
- ☐ The ability to sit in a wheelchair inside your van
- ☐ Brake and/or accelerator on the steering column
- ☐ Other, namely: .....
- ☐ Other, namely: .....
- ☐ Other, namely: .....

Do you use aids when performing certain activities?

- ☐ No, I don't use any aids

Yes, I use (multiple responses are allowed):

- ☐ A crutch (1 crutch / cane)
- ☐ A pair of crutches (two)
- ☐ A wheelchair
- ☐ A walker
- ☐ Other, namely: .....
- ☐ Other, namely: .....
- ☐ Other, namely: .....

Thank you for completing the questions on activities. To finish this questionnaire, please provide us with some personal information in the box below. The information you provide will be handled strictly confidentially.

Today's date : .....

Your date of birth : .....

What type of hemophilia do you have?

Hemophilia type* ☐ 1 Hemophilia A
☐ 2 Hemophilia B

Severity* ☐ 1 Mild
☐ 2 Moderate
☐ 3 Severe

* Please mark the appropriate box

Thank you very much for your cooperation

14.2 HAEM-A-QOL QUESTIONNAIRE

Subject ID: _____

Visit Name: _____

Completion Date: _____
(dd / mmm / yyyy)

HAEM-A- QOL

Questionnaire for Adults

Dear Patient,

We would like to find out how you have been feeling during the past weeks. Please answer the following questions in this questionnaire, which was designed specifically for people with hemophilia.

Please follow the instructions below when answering the questions:

- ⇨ Please read each question carefully.
- ⇨ Think about how things have been for you over the past weeks.
- ⇨ Put an "X" in the box corresponding to the answer that fits you best.
- ⇨ Only mark one box for each question.
- ⇨ There are no right or wrong answers.
- ⇨ It's what you think that matters.
- ⇨ There are some aspects that might not concern you (Sports & Leisure, Family Planning, Work & School, e.g., if you don't work or don't go to school). In such a case, please mark the answer category "not applicable."

All your answers will be treated with the strictest confidence!

Page 2/7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

1. Here we would like to find out about hemophilia and your PHYSICAL HEALTH

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. ... my swellings hurt	☐	☐	☐	☐	☐
2. ... I had pain in my joints	☐	☐	☐	☐	☐
3. ... it was painful for me to move	☐	☐	☐	☐	☐
4. ... I had difficulty walking as far as I wanted to	☐	☐	☐	☐	☐
5. ... I needed more time to get ready because of my condition	☐	☐	☐	☐	☐

2. and now about how you have been FEELING because of your hemophilia

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. ... my hemophilia was a burden for me	☐	☐	☐	☐	☐
2. ... my hemophilia made me angry	☐	☐	☐	☐	☐
3. ... I was worried because of my hemophilia	☐	☐	☐	☐	☐
4. ... I felt excluded	☐	☐	☐	☐	☐

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Page 3/7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

3. How does hemophilia affect your VIEW OF YOURSELF?

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. ... I envied healthy people my age	☐	☐	☐	☐	☐
2. ... I felt comfortable with my body	☐	☐	☐	☐	☐
3. ... hemophilia made my life more difficult	☐	☐	☐	☐	☐
4. ... I felt different from others because of my hemophilia	☐	☐	☐	☐	☐
5. … I was able not to think all the time about my hemophilia	☐	☐	☐	☐	☐

4. These questions are about SPORTS AND LEISURE

In the past 4 weeks...	never	rarely	some-times	often	all the time	not applicable
1. ... I had to avoid sports that I like because of my hemophilia	☐	☐	☐	☐	☐	☐
2. ... I had to avoid sports like football	☐	☐	☐	☐	☐	☐
3. … I played sports just as much as others	☐	☐	☐	☐	☐	☐
4. … I didn't have the freedom to travel where I wanted	☐	☐	☐	☐	☐	☐
5. … it was necessary for me to plan everything in advance	☐	☐	☐	☐	☐	☐

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Page 4/7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

5. These questions are about WORK AND SCHOOL

In the past 4 weeks...	never	rarely	some-times	often	all the time	not applicable
1. ... I was able to go to work/school regularly in spite of my hemophilia	☐	☐	☐	☐	☐	☐
2. … I was able to work/study like healthy colleagues	☐	☐	☐	☐	☐	☐
3. … my everyday work/school activities were jeopardized by my hemophilia	☐	☐	☐	☐	☐	☐
4. ... I found it difficult to pay attention at work/school because I was in pain	☐	☐	☐	☐	☐	☐

6. The next questions are about DEALING WITH HEMOPHILIA

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. … I tried to recognize early on when a bleed developed	☐	☐	☐	☐	☐
2. ... I was able to tell whether or not I was bleeding	☐	☐	☐	☐	☐
3. … I was able to control my bleeds	☐	☐	☐	☐	☐

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Page 5/ 7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

7. and what about your TREATMENT?

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. ... I was dependent on the factor concentrate because of my hemophilia	☐	☐	☐	☐	☐
2. ... I was dependent on physicians for the treatment of my hemophilia	☐	☐	☐	☐	☐
3. ... I was annoyed about the amount of time spent having the injections	☐	☐	☐	☐	☐
4. ... I felt the injections interrupted my daily activities	☐	☐	☐	☐	☐
5. ... I was afraid of complications	☐	☐	☐	☐	☐
6. ... I had problems with how my treatment was administered	☐	☐	☐	☐	☐
7. ... I was afraid that in case of emergency, other doctors wouldn't know how to treat hemophilia	☐	☐	☐	☐	☐
8. ... I was satisfied with the hemophilia center	☐	☐	☐	☐	☐

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Page 6/ 7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

8. What do you think about the FUTURE?

Recently...	never	rarely	sometimes	often	all the time
1. ... I have been thinking that it will be difficult for me to lead a normal life	☐	☐	☐	☐	☐
2. ... I have been expecting that things will get better in the future	☐	☐	☐	☐	☐
3. ... I have been worrying that my condition is worsening	☐	☐	☐	☐	☐
4. ... my life plans have been influenced by my hemophilia	☐	☐	☐	☐	☐
5. ... I have been afraid that I will need a wheelchair	☐	☐	☐	☐	☐

9. The next questions are about hemophilia and your FAMILY PLANNING

Recently...	never	rarely	some-times	often	all of the time	not applicable
1. ... I have had difficulties having children	☐	☐	☐	☐	☐	☐
2. ... I have been afraid that I cannot have children	☐	☐	☐	☐	☐	☐
3. ... I have been afraid that I will not be able to take care of my children	☐	☐	☐	☐	☐	☐
4. ... I worry about not being able to raise a family	☐	☐	☐	☐	☐	☐

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Page 7/ 7 **Visit Name:**_____

Subject ID: _____ **Today's Date:** _____
(dd / mmm / yyyy)

10. What about PARTNERSHIP AND SEXUALITY?

Recently...	never	rarely	sometimes	often	all the time
1. ... I have been finding it difficult to date because of my hemophilia	☐	☐	☐	☐	☐
2 ... I have been insecure in my intimate relationships because of my hemophilia	☐	☐	☐	☐	☐
3. … I haven't been able to have a normal relationship because of my hemophilia	☐	☐	☐	☐	☐

THANK YOU FOR YOUR ASSISTANCE!

14.3 EQ-5D-5L QUESTIONNAIRE

Health Questionnaire

English version for the USA

Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY

I have no problems walking ❑
I have slight problems walking ❑
I have moderate problems walking ❑
I have severe problems walking ❑
I am unable to walk ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

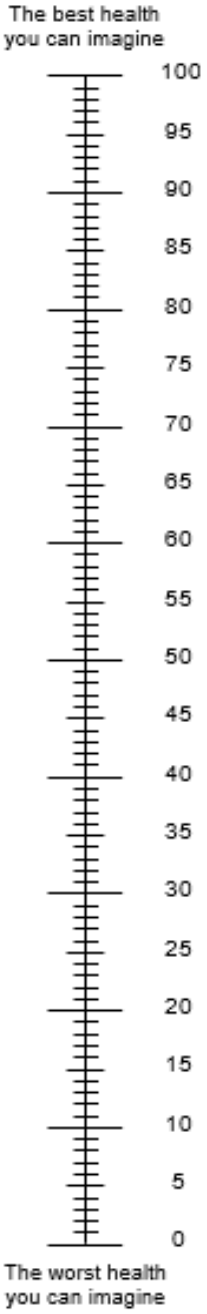
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



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14.4 HEMOPHILIA JOINT HEALTH SCORE

Assessment #: _____ Evaluator Name: _____

Hemophilia Joint Health Score Worksheet 2.1

Subject ID #: _____ Date of Evaluation: _____ yyyy / mm / dd

SWELLING	Left Elbow	Right Elbow	Left Knee	Right Knee	Left Ankle	Right Ankle
None (N), Puffy(P), Spongy(S), Tense(T)	☐N ☐P ☐S ☐T	☐N ☐P ☐S ☐T	☐N ☐P ☐S ☐T	☐N ☐P ☐S ☐T	☐N ☐P ☐S ☐T	☐N ☐P ☐S ☐T
Landmarks: Visible(V); Partially Visible(PV); Not Visible(NV)	☐V ☐PV ☐NV	☐V ☐PV ☐NV	☐V ☐PV ☐NV	☐V ☐PV ☐NV	☐V ☐PV ☐NV	☐V ☐PV ☐NV
Palpable(P); Not Palpable(NP)	☐P ☐NP	☐P ☐NP	☐P ☐NP	☐P ☐NP	☐P ☐NP	☐P ☐NP
SCORE	☐	☐	☐	☐	☐	☐

0 = No swelling
1 = Mild - appears, feels slightly swollen: landmarks visible
2 = Moderate - looks swollen, feels spongy: some landmarks partly obscured
3 = Severe - looks very swollen; is tense: bony landmarks fully obscured

Comments: Please provide any comments in the space provided (If necessary may note circumference in cm)						
DURATION OF SWELLING Note number of months	**Left Elbow**	**Right Elbow**	**Left Knee**	**Right Knee**	**Left Ankle**	**Right Ankle**
Please checkmark one ☐ Patient Report ☐ Parent Report ☐ Reported from chart ☐ Other: _____	____ mths	____ mths	____ mths	____ mths	____ mths	____ mths
SCORE	☐	☐	☐	☐	☐	☐

0 = No swelling or < 6 months
1 = ≥ 6 months

Assessment #: Evaluator Name:_____

Hemophilia Joint Health Score Worksheet 2.1

Subject ID #: _____ Date of Evaluation: _____
yyyy / mm / dd

	Left Elbow	Right Elbow	Left Knee	Right Knee	Left Ankle	Right Ankle
MUSCLE ATROPHY						
SCORE	☐	☐	☐	☐	☐	☐
	0 = **None** - no atrophy **1** = **Mild** - muscle has slighlty less contour, or mild flattening of muscle belly is noted **2** = **Severe** - moderate/severe muscle wasting and depression or flattening of the muscle belly is noted					
Comments: Please note decreased contour, muscle flattening, marked wasting.						
CREPITUS ON MOTION Note: Audible (A) Mild (M) Palpable (P) Severe (S) If none apply: None (N)	**Left Elbow** ☐ A ☐ P ☐ M ☐ S ☐ N	**Right Elbow** ☐ A ☐ P ☐ M ☐ S ☐ N	**Left Knee** ☐ A ☐ P ☐ M ☐ S ☐ N	**Right Knee** ☐ A ☐ P ☐ M ☐ S ☐ N	**Left Ankle** ☐ A ☐ P ☐ M ☐ S ☐ N	**Right Ankle** ☐ A ☐ P ☐ M ☐ S ☐ N
SCORE	☐	☐	☐	☐	☐	☐
	0 = **No** crepitus **1** = **Mild** - slightly audible and/or palpable **2** = **Severe** - Consistently moderately or very pronounced audible and/or palpable grinding and crunching					

Assessment # :

Evaluator Name: _____

Hemophilia Joint Health Score Worksheet 2.1

Subject ID #: _____

Date of Evaluation: _____
yyyy / mm / dd

FLEXION LOSS	Left Elbow	Right Elbow	Left Knee	Right Knee	Left Ankle	Right Ankle
Note Range of Motion (ankle record from 90° starting point)	Flex:	Flex:	Flex:	Flex:	PlantarFlex:	PlantarFlex:
	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐
	The recommendation is to score using both methods (normal contralateral side and normative tables) and then record the worse score.					
SCORE	☐	☐	☐	☐	☐	☐
	Contralateral Side:	**0** = < 5° **1** = Loss of 5° - 10°	**2** = Loss of 11° - 20° **3** = Loss of > 20°	**Normative Tables:**	**0** = Within Range **1** = Loss of 1 to 4°	**2** = Loss of 5° - 10° **3** = Loss of > 10°

EXTENSION LOSS	Left Elbow	Right Elbow	Left Knee	Right Knee	Left Ankle	Right Ankle
Note Range of Motion (ankle record from 90° starting point)	Ext:	Ext:	Ext:	Ext:	DorsiFlex:	DorsiFlex:
Hyperextension: record as "plus" (+) _____ degrees	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐	Measured in: 1) Supine ☐ 2) Sitting ☐
Loss of extension record as "minus" (-) _____ degrees	The recommendation is to score using both methods (normal contralateral side and normative tables) and then record the worse score.					
SCORE	☐	☐	☐	☐	☐	☐
	Contralateral Side:	**0** = < 5° **1** = Loss of 5° - 10°	**2** = Loss of 11° - 20° **3** = Loss of > 20°	**Normative Tables:**	**0** = Within Range **1** = Loss of 1 to 4°	**2** = Loss of 5° - 10° **3** = Loss of > 10°

Assessment #: **Evaluator Name:** _____

Hemophilia Joint Health Score Worksheet 2.1

Subject ID #: _____ **Date of Evaluation:** _____
yyyy / mm / dd

JOINT PAIN Active joint mov't through range with gentle pressure (at end range)	**Left Elbow** Comments:	**Right Elbow** Comments:	**Left Knee** Comments:	**Right Knee** Comments:	**Left Ankle** Comments:	**Right Ankle** Comments:
SCORE	☐	☐	☐	☐	☐	☐
	0 = No pain through active range of motion **1** = No pain through active range; only pain on gentle overpressure or palpation **2** = Pain through active range					

STRENGTH Using the Daniels & Worthingham's scale. Within available ROM, note grade	**Left Elbow** Flexion Extension	**Right Elbow** Flexion Extension	**Left Knee** Flexion Extension	**Right Knee** Flexion Extension	**Left Ankle** # of heel raises_____ PlantarFlex. DorsiFlex.	**Right Ankle** # of heel raises_____ PlantarFlex. DorsiFlex.
SCORE	☐	☐	☐	☐	☐	☐
	0 = Holds test position against gravity with maximum resistance (gr.5) **1** = Holds test positon against gravity with moderate resistance (but breaks with maximal resistance) (gr.4) **2** = Holds test position against gravity with minimal resistance (gr. 3+), or holds test position against gravity (gr.3) **3** = Able to partially complete ROM against gravity (gr.3-/2+), or able to move through ROM gravity eliminated (gr.2), or through partial ROM gravity eliminated (gr.2-) **4** = Trace (gr.1) or no muscle contraction (gr.0) **NE** = Non-evaluable				**# of Heel Raises:** (to be used only for plantarflexion scoring) **Score 0** = 4 to 5 heel raises **Score 1** = 2 to 3 heel raises **Score 2** = Sufficiently plantar flexes to clear heel **Score 3** = Plantar flexes ankle through range (gravity elimnated) **Score 4** = trace or no muscle contraction	

2013-02-25

15 APPENDIX 3: ABBREVIATIONS

AAV2	Adeno-associated virus vector, serotype 2
AAV5	Adeno-associated virus vector, serotype 5
[redacted]	[redacted]
AAV8	Adeno-associated virus vector, serotype 8
AAVhu37	Adeno-associated virus vector, serotype hu37
AAVrh10	Adeno-associated virus vector, rhesus serotype 10
[redacted]	[redacted]
[redacted]	[redacted]
ABR	Annualized bleeding rate
AE	Adverse event
AESI	Adverse event of special interest
AFU	Annualized factor usage
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
Anti-HBc	Total Hepatitis B core antibody
APRI	AST-to-Platelet Ratio Index
aPTT	Activated partial thromboplastin time
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase
[redacted]	[redacted]
[redacted]	[redacted]
BID	Twice daily
BMI	Body mass index
[redacted]	[redacted]
bp	Base pair
BP	Blood pressure
BU	Bethesda units

BUN	Blood urea nitrogen
BQL	Below Quantifiable Limits
˚C	Celsius
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CDC	U.S. Centers for Disease Control and Prevention
cDNA	Complimentary deoxyribonucleic acid
CIOMS	Council for International Organizations of Medical Sciences
Cl	Chlorine
CL	Central laboratory
C_{max}	Maximum drug concentration
CNS	Central nervous system
COVID-19	Coronavirus disease caused by SARS-CoV-2
CRP	C-reactive protein
CQA	Clinical Quality Assurance
CTA	Clinical trial agreement
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T lymphocyte
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
EASL	European Association for the Study of the Liver
EC	Empty capsid
ECG	Electrocardiogram
CRF/eCRF	Case report form/electronic case report form
ED	Exposure day
EDC	Electronic Data Capture
EHL	Extended half-life
ELISpot	Enzyme-linked immunospot assay
EOS	End of study
EQ-5D-5L	Euro quality-of-life five dimensions questionnaire
FAS	Full analysis set
FDA	U.S. Food and Drug Administration

FIX	Coagulation factor IX
FIXa	Activated factor IX
FVIII	Coagulation factor VIII
FVIII:C	Factor VIII in circulation
[redacted]	[redacted]
FXa	Activated factor X
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HAART	Highly active anti-retroviral therapy
Haem-A-QoL	Hemophilia Quality of Life Questionnaire
HAL	Hemophilia activities list
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High-density lipoprotein
hFIX39-Padua	Human coagulation factor IX Padua variant
hFVIII	Human coagulation factor VIII
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HR-QoL	Health-related quality-of-life
IB	Investigator's Brochure
ICF	Informed consent form
ICH	Intracranial hemorrhage
ICH GCP	International Conference on Harmonization Good Clinical Practice
ID	Identification
IEC	Institutional Ethics Committee
IFN-γ	Interferon gamma

IgG	Immunoglobulin G
IgG4	Immunoglobulin G subclass 4
IgM	Immunoglobulin M
IL	Interleukin
ILI	Influenza like illness
IND	Investigational new drug application
IRB	Institutional Review Board
ITR	Inverted terminal repeats
IU	International units
IV	Intravenous
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LFT	Liver function test
LL	Local laboratory
LPLD	Lipoprotein lipase deficiency
LPLV	Last Participant's Last Visit
LSEC	Liver sinusoidal endothelial cells
LTFU	Long-term follow-up
MERS-CoV	Middle East respiratory syndrome-related coronavirus
MHC	Major histocompatibility complex
[redacted]	[redacted]
mRNA	Messenger ribonucleic acid
NAb	Neutralizing antibody
NAAT	Nucleic acid amplification testing
NCT	National Clinical Trial
NHF-MASAC	National Hemophilia Foundation's Medical and Scientific Advisory Council
NHP	Non-human primate
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PHI	Protected health information
PI	Principal Investigator

PK	Pharmacokinetics
PML	Progressive multifocal leukoencephalopathy
PV	Pharmacovigilance
QoL	Quality of life
rAAV	Recombinant adeno-associated viral vectors
RBC	Red blood cells
rFVIII	Recombinant factor VIII
RNA	Ribonucleic acid
RPE65	Retinal pigment epithelium 65 gene
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SPK-8016	Study drug in this protocol [redacted]
SPK-9001	[redacted]
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
[redacted]	[redacted]
TEE	Thrombotic and/or embolic events
TNFα	Tumor Necrosis Factor alpha
TPMT	Thiopurine methyltransferase
TTR	Transthyretin
TTRm	Modified transthyretin
ULN	Upper limit of normal
URTI	upper respiratory tract infection
vg	Vector genome
VLDL	Very low-density lipoprotein
VWF	von Willebrand factor
WBC	White blood cell

WFH	World Federation of Hemophilia
WHO	World Health Organization
XLMTM	X-linked myotubular myopathy

16 APPENDIX 4: SUMMARY OF CHANGES FROM THE PREVIOUS PROTOCOL VERSION

AMENDMENT 2 SECTION	SUMMARY OF REVISIONS MADE	RATIONALE
1.1 Protocol Synopsis	Protocol Synopsis has been updated.	Updates reflect changes made throughout Amendment 2.
Study Schema Figure	Updated Study Schema Figure 1.	The study schematic was updated to include the two cohorts.
Schedule of Assessments	Updated Schedule of Assessments Table 1.	Additional screening and follow up is required. This includes screening labs, three to seven days prior to vector, Day -2 infusion of [redacted] and weekly follow up while on immunomodulation. In addition, the FVIII levels on Day 0 were eliminated for all participants.
2.2.4 & 2.2.5	Added Summary of Non-clinical Experience with [redacted]	The preclinical study results provide evidence to support the design of cohort.
2.2.5	Added preclinical experience with [redacted]	The preclinical study results provide evidence to support the design of cohort 1.
2.3.1	Added risks associated with [redacted]	The risks associated with [redacted] were provided to support the design of cohort 2.
2.3.1	Added risks associated with [redacted]	The risks associated with [redacted] were provided to support the design of cohort 1
2.4	Updated Study Rationale with most recent published data.	Results from gene therapy in hemophilia have been published since the previous amendment. Data from the Spark participants to date provides the rationale for this amendment.
4.1.1	Added cohorts 1 and 2 to the study design and provided instruction on sequence of enrollment.	Details for the expansion and enrollment sequence of the two cohorts are included. A description of the expansion of cohorts up to

		10 participants is described if additional data is needed.
4.1.1	Refined dose cohort expansion.	Details for the expansion and enrollment sequence of the 3 doses into two cohorts are included. A description of the expansion of cohorts up to 10 participants is described if additional data is needed.
4.1.3.1	Added SARS-coV2 testing	Multiple effects of COVID-19 on the hemostatic system have been described, including the development of anti-phospholipid antibodies (Lupus-like anticoagulants), which might confound interpretation of one-stage clotting factor VIII assays
4.2.2	Updated study design to include up to 40 participants.	The addition of one additional dose and expansion of all dose levels into two cohorts will require an increase in the number of participants in the trial.
5.2	Provided clarification in eligibility for assessing liver fibrosis.	On occasion, discordant results as it relates to liver health have been found in review of the medical record. The Fibro scan is considered the gold standard in determining liver elasticity/fibrosis. This protocol was clarified to elevate this test as the determinant for eligibility.
5.2	Exclusion criteria updated to included gastrointestinal disease, cancer, and latent infectious disease due to the addition of immunomodulation in the amendment.	The use of immunomodulation agents has been included in the study design. The exclusion criteria changes are reflective of contraindications identified in the package insert for [redacted]
6.2.6	Added ability to perform genetic analyses on archived samples	Archived samples may be used for genetic sequencing if the participant provides consent.
6.4.1	Changed the FVIII PK analysis from Day 0 to historic or during the screening period.	The PK analysis added to the complexity of Day 0. Historic PK results provide sufficient information should it be needed to compare FVIII response post gene therapy.

6.7.1	Changed the Day 0 FVIII infusion to a routine prophylactic treatment.	The PK analysis is no longer needed on Day 0. Patients with hemophilia administer prophylactic treatments routinely. This will provide bleed protection for a portion of week one.
6.5 & 6.6	Added additional laboratory assessments for Cohort 2 prior to dosing day and following vector infusion.	Additional laboratory assessments are required for participants in cohort 2 due to the planned use of [redacted] The testing three to seven days prior to dosing day is required to confirm that the critical laboratory values have not changed during the screening period. Weekly follow up while on immunomodulation is recommended in the clinical management of participants receiving immunomodulation. NAAT testing for infection with SARS-coV2 in asymptomatic study participants prior to immune suppression and to recommend NAAT testing in symptomatic study participants prior to immune suppression has been included for participant safety.
7	Added information on description, storage, preparation, handling, disposal, accountability, and labeling of Sponsor-provided immunomodulators	Language was added to support Sponsor supply of immunomodulators.
9.1.1	Updated AE language	AE language was updated to include standard toxicology reporting. Clarification of the timeframe to collect AEs was provided.
11.1.9	Added requirements for eCRF data to be locked before a site is closed.	Clarification was provided to ensure eCRF data for a site is locked prior to that site being closed.
Throughout	Updated text and references as they relate to the Immunomodulatory clinical experience and the cohort design in this trial.	Literature in the gene therapy is regularly published. Data and references were updated in all areas of the protocol to maintain relevant resources. Data from the

		Spark participants to date provides the rationale for this amendment.

Document	Description of Main Changes
Amendment 1	1. Incorporated minor edits for readability, corrected formatting issues, and corrected typographical errors to improve accuracy and clarity and updated abbreviations, citations and references. Changes and updates from the Sponsor's latest protocol template were incorporated. 2. Updated study schematic figure 1. 3. Removed language requiring prophylactic steroid administration on Week 4. 4. [redacted] corticosteroids in response to an immune response trigger. 5. Updated language to allow the Investigator in collaboration with the Sponsor to use other immunomodulatory agents, should they be required, to reduce long term and high dose steroid exposure 6. Inserted Thiopurine methyltransferase (TPMT) as a screening requirement. 7. Added risks associated with [redacted]. 8. Updated text and references as it relates to immunomodulatory clinical experience in Hemophilia Gene Therapy trials.