TITLE PAGE

PROTOCOL TITLE:	Gene-transfer, open-label, dose-escalation study of SPK-8011 [adeno-associated viral vector with B-domain deleted human factor VIII gene] in individuals with hemophilia A
PROTOCOL NUMBER:	SPK-8011-101
INVESTIGATIONAL PRODUCT/NUMBER:	SPK-8011
PHASE OF DEVELOPMENT	1/2a
INDICATION:	Hemophilia A
SPONSOR:	Spark Therapeutics
SPONSOR ADDRESS:	3737 Market Street, Suite 1300 Philadelphia, PA 19104
REGULATORY AGENCY IDENTIFYING NUMBER(S):	IND #17151
ORIGINAL PROTOCOL DATE:	17-Oct-2016
AMENDMENT NUMBER:	7
VERSION DATE:	09-Feb-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: Gene-transfer, open-label, dose-escalation study of SPK-8011 [adeno-associated viral vector with B-domain deleted human factor VIII gene] in individuals with hemophilia A

Protocol No: SPK-8011-101

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:

Head of Clinical Research and Development or Medical Designee that Signed the Internal Protocol Approval Form:

PPD

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 me and my staff 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 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.

Principal Investigator (PI) (signature) Date (dd-MMM-yyyy)

Principal Investigator (PI) Name (printed) Site Number

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.

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: Gene-transfer, open-label, dose-escalation study of SPK-8011 [adeno-associated viral vector with B-domain deleted human factor VIII gene] in individuals with hemophilia A

Protocol Number: SPK-8011-101

Sponsor: Spark Therapeutics

Development Phase: 1/2a

Name of Investigational Product: SPK-8011

Study Indication: Hemophilia A

Rationale: There is no available cure for hemophilia A. Natural history data in individuals with hemophilia A suggest that circulating factor levels approximately 12% of normal may be sufficient to protect against spontaneous joint bleeds (den Uijl, 2011). A vector that can safely and consistently achieve sustained high levels of FVIII activity, which could potentially eliminate spontaneous hemarthroses, is the goal of gene transfer in hemophilia treatment.

The objective of this study is to determine the safety, tolerability, and efficacy (transgene-derived FVIII activity levels) of a single intravenous (IV) infusion of SPK-8011 in individuals with severe hemophilia A.

The recent results from an AAV5-mediated B-domain-deleted human factor VIII (AAV5-BDD-hFVIII) BMN-270 gene transfer trial for severe hemophilia A resulted in sub-therapeutic levels (0.6% FVIII activity levels) over 34 weeks in 1 participant at $6x10^{12}$ vg/kg (low dose-level), transgene FVIII activity levels 2.3% of normal over 26 weeks in 1 participant at $2x10^{13}$ vg/kg (middle dose-level), and lastly, FVIII activity levels 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 show FVIII within the

normal range, and 1 out of 7 in the range of 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 of 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 the vector administration. These 6 participants given prophylactic corticosteroids had exposure from 15 weeks to at least 35 weeks in 1 participant (Pasi, 2020).

As of 24 November 2020, in this SPK-8011-101 study, participants have received SPK-8011 at dose levels $5x10^{11}$ vg/kg, $1x10^{12}$ vg/kg, $2x10^{12}$ vg/kg, and $1.5x10^{12}$ vg/kg. In attempts to avoid the loss of factor VIII transgene expression, both reactive and early prophylactic approaches to corticosteroid use have been studied. The reactive treatment with corticosteroids, generally initiated ~ Weeks 7-11 following vector administration, was performed with a relatively limited course of prednisone. In contrast to this, when steroids have been initiated at approximately 4 weeks after vector administration with a goal of prophylactic maintenance of FVIII expression, prolonged tapering of corticosteroids has been required to avoid and treat late recurrences of apparent hepatocyte-targeted immune response. In the case of some patients with a prolonged steroid taper, it has been clinically necessary to initiate a steroid sparing regimen(s) (e.g., azathioprine, tacrolimus) to aid in the prednisone taper.

Additional investigation is 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-8011 beginning at the $1.5x10^{12}$ vg/kg dose will be explored in 2 cohorts, based on prior clinical studies, non-clinical studies summarized in protocol introduction, and input from clinical experts in rheumatology, organ transplantation, viral immunology, and bone marrow transplantation. Cohorts 1 and 2 will receive immunomodulation with mycophenolate mofetil (MMF) or tocilizumab (TCZ), respectively, 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 factor VIII (FVIII). IV CCI steroids will be preferentially used when clinical and other circumstances permit. The ELISpot will not be considered a predictive tool for clinical

decision making independent of other immune triggers. Oral corticosteroids may be used when clinically indicated. CCI

[redacted]

Study Design:

This 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-8011 in adult men with severe hemophilia A (as defined in the inclusion criteria). Up to 50 eligible participants will be dosed with a single IV infusion of SPK-8011.

Participants will provide informed consent and undergo screening assessments up to 16 weeks prior to SPK-8011 infusion on the Dosing Day (Day 0). All participants will be monitored for a total of 52 (±2) weeks (End of Study) after SPK-8011 infusion (see Schedule of Events).

Week 52 procedures are to be performed only at the EOS Visit. If the long-term follow-up (LTFU) study is not open at the clinical site at the time of a participant's planned Week 52 visit, the participant may remain in Study SPK-8011-101. 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-8011. • To evaluate the efficacy of SPK-8011.
Secondary
• To determine the PK characteristics of SPK-8011.

	• To characterize the immune response to the vector and transgene product.
	Endpoints
	Primary

	• For safety and tolerability:
	  ○ Clinically notable changes from baseline in physical examinations and vital signs.
	  ○ Incidence of adverse events, including clinically significant abnormal laboratory values.
	  ○ Hepatic transaminase elevation requiring immunosuppression.
	• For efficacy:
	  ○ Primary kinetic parameters of peak and steady-state FVIII activity levels assessed by coagulation clotting assays.
	  ○ Number of FVIII infusions after vector administration.
	  ○ Number of bleeding events (spontaneous and traumatic) after vector administration.

	Secondary

	• Additional kinetic assessments will include, but not limited to:
	  ○ Time to achieve steady-state FVIII activity level.
	  ○ Vector shedding of SPK-8011 in bodily fluids.
	• Incidence of immune response 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 PBMCs after vector administration (ELISpot, and other exploratory biomarkers)
Study Population:	This study will be conducted in males with severe hemophilia A (as defined in the inclusion criteria) who are ≥ 18 years of age.
Inclusion/Exclusion Criteria:	Participants must meet the following criteria at screening and prior to dosing of SPK-8011 (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 (EDs) to any recombinant and/or plasma-derived (pd) FVIII protein products or cryoprecipitates based on historical data from the medical records/history;
5. Have no prior history of hypersensitivity or anaphylaxis associated with any FVIII or IV immunoglobulin administration;
6. 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 remote (greater than 5 years) documentation 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 ≤ upper limit of normal (ULN) at the testing laboratory;
    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 TCZ in Cohort 2
8. Agree to use reliable barrier contraception after the administration of SPK-8011 until notified by the Investigator or designee.

If any of the following exclusion criteria exist at screening or prior to dosing of SPK-8011 (Day 0), participants 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 at screening for each of the following tests: HBsAg (hepatitis B surface antigen), anti-HBc (total hepatitis B core antibody), and a 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. Participants treated with anti-viral therapy for chronic hepatitis C must have completed anti-viral therapy at least 6 months prior to screening and have a negative HCV-RNA at the time of screening.
        iii. Participants with a documented or self-reported history of HCV must have a single negative HCV-RNA at time of screening.
2. 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:

3a. Serum albumin measurement. A participant is not eligible if the serum albumin level is below the lower limit of normal of the laboratory; ***and***

3b. A 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. AST-Platelet Ratio Index (APRI) > 1

If more than 1 diagnostic test result is available, then the FibroScan 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. Participants who are HIV-positive and stable, with an adequate $CD4^+$ count (> 200/mm^3) and undetectable viral load (< 50 gc/mL) measured twice in the 6 months prior to enrollment, and who are on an antiretroviral drug regimen are eligible to enroll;
5. Have anti-AAV-Spark200 neutralizing antibody titers ≥ 1:1;
6. Have 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** participated in a clinical study with an investigational drug within the last 12 weeks prior to signing the informed consent;
8. History of diverticulitis, diverticulosis requiring antibiotic treatment, or chronic ulcerative lower gastrointestinal disease that might predispose a patient to perforations;
9. Any concurrent clinically significant major disease (such as liver abnormalities, type I diabetes, uncontrolled hypertension, vertebral compression) or 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. 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 consideration of the potential clinical relevance of additional screening;

10. Planned surgical procedure in the next 12 months requiring FVIII prophylactic treatment; and
11. 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 (±2) weeks, including screening. The study will include the following phases:

a) Screening period (up to a maximum of 16 weeks);
b) Dosing day (Day 0);
c) Follow-up observation period [52 (±2) weeks].

Planned Number of Participants: Up to 50 evaluable participants will be dosed with a single intravenous infusion of SPK-8011.

Number and Location of Study Sites: The study is planned to be conducted at approximately 20 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-8011 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-8011 at a given dose level prior to infusing the first participant in the next dose level.
c) Continued enrollment will alternate between Cohorts 1 and 2 beginning at $1.5x10^{12}$ vg/kg. A stagger of at least 6 weeks will occur between the first and the second participants in Cohort 2 (see Figure 1).
d) HIV positive individuals with stable $CD4^+$ count > 200 mm^3 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 assessments will include 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 parameters changes over time, and the use of any immunosuppressive therapy.

The infusion of SPK-8011 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-8011 will consist of peak and steady-state values of circulating vector-derived FVIII activity levels after SPK-8011 infusion. Additional pharmacokinetic (PK) evaluation of SPK-8011 is carried out by analysis of serum, urine, semen, saliva, and peripheral blood mononuclear cells (PBMCs) 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 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:

SPK-8011 is a recombinant adeno-associated viral (rAAV) vector designed to drive expression of a B-domain deleted human factor VIII (BDD-hFVIII) transgene and raise the circulating activity levels of endogenous FVIII. SPK-8011 is comprised of a bio-engineered rAAV capsid (AAV-Spark200) and an expression cassette encoding B-domain-deleted, codon-optimized human FVIII (hFVIII). Dose-ranging studies in non-human primates (NHPs) infused with SPK-8011 have shown average hFVIII antigen levels that peaked approximately 2 to 3 weeks after infusion, with levels ranging from 22.3 ± 6.2% of normal, with a dose of $2x10^{12}$ vg/kg, to 153 ± 58.1% of normal,

with highest dose of $2x10^{13}$ vg/kg without observed safety concerns. More recently, chimeric 'humanized' mice with livers partially repopulated with human hepatocytes have become available as a tool to determine hepatic transduction efficacy of different viral capsids. CCI [redacted] Thus, the Sponsor suggests an initial starting dose level of $5x10^{11}$ vg/kg, assuming a linear extrapolation of FVIII levels in humans to be approximately 3% of normal.

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-

Dose-level expansion may occur under the following scenarios:

A. ***Dose-Level Expansion*** **-** After 2 participants are dosed in a given dose-level, it may be possible to expand a dose-level 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*** **–** 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 ($5.0x10^{11}$ 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:

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.

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 and without use of exogenous FVIII product since vector administration.

After escalation through the first 3 planned dose levels, an intermediate dose level (e.g., $1.5x10^{12}$ vg/kg of SPK-8011), was agreed upon by 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 cohort ($2x10^{12}$ vg/kg) to better evaluate the safety, efficacy, and variability of response within a given cohort. Any decision to add an intermediate 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 less than 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 regular basis during the course of the study. The specifics regarding the DMC organization and procedures will be outlined in the DMC Charter.

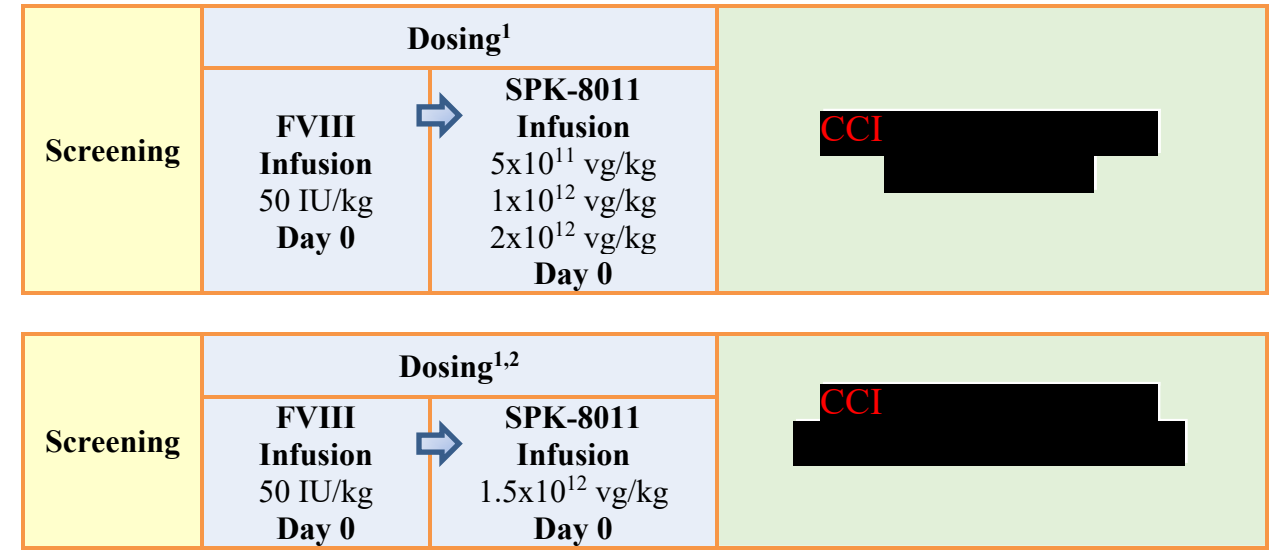
Statistical Methods:

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-8011 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 displayed using counts and percentages within each category.

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

1.2 Schema

Figure 1 Study Schematic



1.3 Schedule of Events

Tests and Assessments	Screen[1] Weeks -16 to -1	3 to 7 Days prior to Day 0	CCI	Day 0				Day 1	Week 1 – 16 (±2 days)[3]											Week 18–52 (±2 weeks)[3]							
				FVIII Dosing	CCI	Pre-SPK-8011 Dosing	SPK-8011 Dosing	30 (±2) min, 2 and 5 hrs (±10 min) from stop of SPK-8011 infusion	24 hrs (±1hr)	1		2, 3, 4, 5, 6		7		8, 9, 10, 11		12	14	16	18	22	26	30	34	40	46	52/ EOS[2,23]
										A	B	A	B	A	B	A	B										
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 joint assessment, HJHS																						X					X
Physical exam, height, weight[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																											X
HBsAg, anti-HBc, HBV-DNA (CL)	X[10]																	X[28]									
HCV-RNA load assay (CL)	X[10]																										
HIV-1/HIV-2 Ag/Ab (CL)	X[10]																										
CD4$^+$ count, HIV-1/HIV-2 viral load *(for positive participants only)* (CL)	X[10]																										
Hematology (CL, LL)[25]	X	X[24]							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

Tests and Assessments	Screen[1] Weeks -16 to -1	3 to 7 Days prior to Day 0	CCI	Day 0					Day 1	Week 1 – 16 (±2 days)[3]											Week 18–52 (±2 weeks)[3]							
				FVIII Dosing	CCI	Pre-SPK-8011 Dosing	SPK-8011 Dosing	30 (±2) min, 2 and 5 hrs (±10 min) from stop of SPK-8011 infusion	24 hrs (±1hr)	1		2, 3, 4, 5, 6		7		8, 9, 10, 11		12	14	16	18	22	26	30	34	40	46	52/ EOS[2,23]
										A	B	A	B	A	B	A	B											
Fibrinogen Ag, thrombin time, D-dimer[35] (LL)	X											X																
Coagulation – aPTT and FVIII activity (CL, LL), FVIII Ag (CL)[11]	X					X[17]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation - FVIII inhibitor (CL, and LL at screening), VWF activity and VWF Ag (CL)	X																											X
LFTs and CRP (LL and CL)[11]	X	X[24]							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunology (CL)		X[24]				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)* (LL, CL)[14,25]	X																											X
AAV neutralizing Ab (CL)[31]	X									X		X		X				X	X				X			X		X
Vector shedding (CL)[15]										X		X		X		<<< *if indicated* >>>												
Immune profiling (CL)[29,32]		X[24]				X		X	X		X		X				X			X								
PAX gene (RNA) (CL)[30,32]		X				X		X	X				X				X											
Spare plasma (CL)[21]	X								X									X					X			X		X
TPMT (LL)	X																											
TB testing (LL)	X																											
SARS-CoV2 testing (LL)[33]	X	X																		X								

Tests and Assessments	Screen[1] Weeks -16 to -1	3 to 7 Days prior to Day 0	CCI	Day 0	CCI				Day 1	Week 1 – 16 (±2 days)[3]										Week 18–52 (±2 weeks)[3]							
				FVIII Dosing		Pre-SPK-8011 Dosing	SPK-8011 Dosing	30 (±2) min, 2 and 5 hrs (±10 min) from stop of SPK-8011 infusion	24 hrs (±1hr)	1		2, 3, 4, 5, 6		7		8, 9, 10, 11		12	14	16	18	22	26	30	34	40	46	52/ EOS[2,23]
										A	B	A	B	A	B	A	B										
Infusion with FVIII product[16]				X																							
Infusion with SPK-8011[18]							X																				
CCI																											
Hemophilia Activities List, Health-economic Assessment[19]	X													X				X	X				X			X	X
Haem-A-QoL, EQ-5D-5L[19]	X[6]																						X				X
PK profile				X[34]																							
Dispense/review/collect FVIII Infusion Log[20]	X					X								X				X	X				X			X	X
Adverse events	X					*ongoing*																					
Prior and concomitant therapies/procedures	X					*ongoing*																					
Unscheduled visit or safety test[22]									*if indicated*																		

AAV = adeno-associated virus, Ab = antibody, Ag = antigen, ALT = alanine aminotransferase, aPTT = activated partial thromboplastin time, AST = aspartate aminotransferase, CL = central lab (refer to Laboratory Manual), $CD4^+$ = cluster of differentiation 4, CRP = C-reactive protein, CTL = cytotoxic T-lymphocyte, ECG = electrocardiogram, EOS = end of study, EQ-5D-5L = EuroQol- 5 Dimension 5 Level, FVIII = coagulation factor VIII, GGT = gamma-glutamyl transferase, Haem-A-QoL = Haemophilia A Quality of Life, HBc = hepatitis B core, HBs = hepatitis B surface, HBV = hepatitis B virus, HCV = hepatitis C virus, HDL = high-density lipoprotein, HIV = human immunodeficiency virus, HJHS = Hemophilia Joint Health Score, HLA = human leukocyte antigen, IV = intravenous, LDH = lactate dehydrogenase, LDL = low-density lipoprotein, LFT = liver function test, LL = local lab, LTFU = long-term follow-up, CCI NAAT = nucleic acid amplification testing, PBMC = peripheral blood mononuclear cells, PCR = polymerase chain reaction, PK = pharmacokinetic, SARS-CoV2 = severe acute respiratory syndrome coronavirus 2, TB = tuberculosis, CCI, TPMT = thiopurine methyltransferase, VLDL = very low-density lipoprotein, VWF = von Willerbrand Factor.

Notes for Schedule of Events:

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. 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, including Weeks 1B-6B and 7B-11B, 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 HLA samples will be collected only if not known. Investigator and participant to review age-appropriate vaccinations during the screening period, and at least 4 weeks prior to planned day of administration of SPK-8011.
6. Assessments are completed between screening visit and prior to Dose Day 0. Joint assessments and lab collection for vector shedding is allowed on Day 0 pre-SPK-8011 Dosing.
7. Height is 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-8011. Physical Exam is comprehensive (not targeted), at all designated visits. Hemophilic arthropathy should be assessed during the physical exam.
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 min at the following timepoints: SPK-8011: Pre-infusion and post-infusion of SPK-8011 (i.e., 30 min (±2 min), 2 hrs (±10 min), and 24 (±1) hrs)
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 LFTs will be performed by CL and LL. Twice weekly monitoring of AST, ALT, GGT, LDH, aPTT, FVIII:C, and FVIII Ag will be performed during Weeks 1 through 11 post vector-infusion visits. LL results may be used for eligibility assessment. LFTs on 3-7 days prior to Day 0 are performed by the local lab only.
12. Screening ECG is required for participant > 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, HDL, LDL, VLDL, triglycerides) will be performed for participants with a history of dyslipidemia or hypercholesterolemia, or if clinically indicated.
15. Vector shedding: PCR analysis will be performed; PBMC, serum, saliva, urine, and semen will be collected between screening and Day 0 (prior to vector infusion) and weekly starting at Week 1 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 CCI 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 participant's Infusion Log. Site assistance is permitted for the FVIII infusion.
17. Day 0 blood samples for FVIII activity, FVIII Ag, and aPTT will be collected pre-SPK-8011 infusion.
18. Participants will receive a single IV infusion of SPK-8011 for approximately 60 (±2) min.
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 screening or Day 0 (pre SPK-8011 infusion), with collection, review, and dispensing at subsequent visits.
21. Spare plasma will be collected for immunology, further coagulation assays, future research, or for clarification of any clinical or laboratory adverse events.

22. Unscheduled visits or safety tests may be performed for safety monitoring purposes or repeat safety assessments.
23. Week 52 procedures are to be performed only at the EOS Visit. If the LTFU study is not open at the clinical site at the time of a participant's planned Week 52 visit, the participant may remain in Study SPK-8011-101. 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.
24. Local hematology, local LFT, 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 CCI
25. Weekly hematology and monthly lipid profile should be monitored locally for Cohorts 1 and 2 until CCI . The frequency may be altered based on clinical response.

CCI

28. Participants in Cohorts 1 and 2 will be retested at 12 weeks for HBsAg and anti-HBc.
29. Samples for immune profiling will be collected CCI pre-administration of SPK-8011, and 30 (±2) min, 2 hrs (±10 min), 5 hrs (±10 min), and 24 (±1) hrs post vector infusion, and Weeks 1B, 2B, 3B, 4B, 8B, and 16.
30. Samples for PAX gene will be collected CCI pre- administration of SPK-8011, 5 hrs (±10 min), and 24 (±1) hrs post vector infusion, and Weeks 2B, 4B, and 8B.
31. Samples for AAV neutralizing antibody will be collected during screening, then Week 1A, Week 2A, Week 4A, Week 7A, then Weeks 12, 14, 26, 40, and 52/EOS
32. If an apparent CTL immune response/liver inflammation is observed and triggers the initiation of reactive corticosteroids, additional samples will be collected for immune profiling and PAX gene at the following times: prior to initiating CC IV corticosteroids or oral corticosteroids (if possible, without delaying the initiation of corticosteroid therapy) and then CCI following initiation of corticosteroids.
33. Anti-SARS-CoV2 serology testing will be done at screening and at Week 18. At the discretion of the investigator, 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 between screening and Day 0.
35. Fibrinogen Ag, thrombin time, D-dimer are required locally at screening, Week 2A, and Week 4A for participants enrolled in Cohort 2.

2 INTRODUCTION

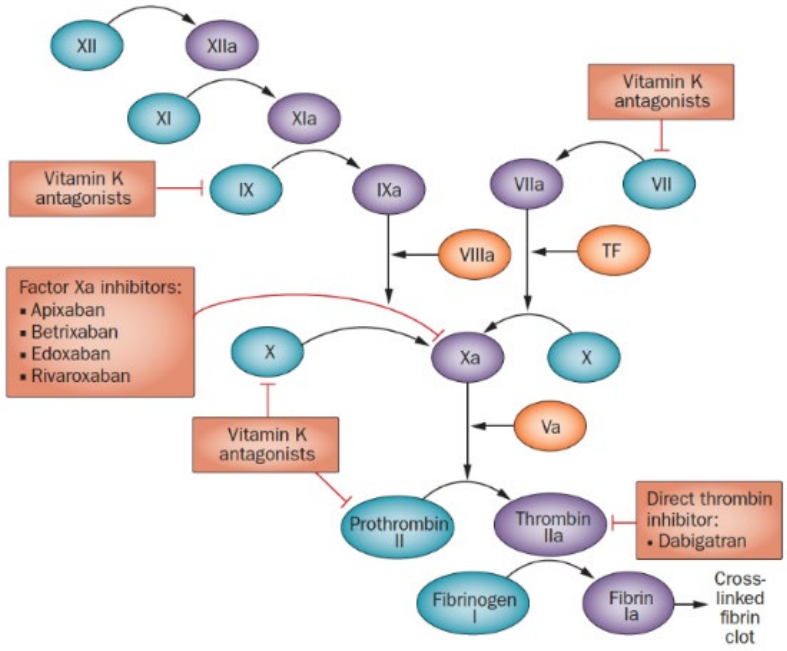
SPK-8011, an investigational gene transfer medicinal product, is a rAAV vector that contains a bioengineered capsid (AAV-Spark200) and a codon-optimized expression cassette to drive expression of human coagulation factor VIII (FVIII). SPK-8011 is being developed by Spark Therapeutics for the treatment of hemophilia A. *The Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products* (U.S. Food and Drug Administration, 2015) and findings from previous clinical studies with AAV2, AAV8 and AAV-Spark100 vectors were considered in the development of this protocol.

2.1 Background

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 factor VIII (FVIII) measured in the plasma. Severe hemophilia A is defined as a coagulation activity of FVIII in plasma (FVIII:C) level of < 1% (< 1 IU/dL) normal levels. Factor VIII, a single chain glycoprotein pro-cofactor, is composed of 6 putative domains arranged in the order of heavy chain (A1, A2, and B regions) and 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 which stabilizes the intrinsically unstable FVIII protein. Observations that the FVIII-VWF complex is dissociated by phospholipid and that VWF prevents FVIII from binding to phospholipid and platelets suggests antagonism between phospholipid and VWF for binding to FVIII. Although FVIII and VWF are glycoproteins, they have different roles in the initiation and regulation of hemostasis. VWF is necessary for mediation of platelet-vessel interactions at the site of vascular injury. Thrombin 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 in 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 but 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. The level of coagulation factors in the plasma of normal individuals ranges from 50 - 150% (or 50 -150 IU/dL) of the level in normal pooled plasma. Therefore, 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 (ICH). 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 (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). ICH 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. ICH 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 (usually immunoglobulin G subclass 4 (IgG4) antibodies that neutralize the procoagulant activity of FVIII or coagulation factor IX (FIX)) to FVIII are estimated to occur in 20-35% 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 inhibitory antibodies, replacement of the missing clotting factor by infusion of FVIII becomes less effective. Once replacement therapy is ineffective, 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 four 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). HCV disease can cause chronic and progressive hepatitis, with eventual development of cirrhosis, and affects the majority of persons with hemophilia A born before 1987. 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, there have been no documented transmissions of hepatitis B and C viruses, HIV, West Nile, or malaria since the introduction of effective virus inactivation procedures (Tabor, 1999). However, there is no pathogen inactivation process that has been shown to eliminate all pathogens (such as rare reports of infectious prions and parvovirus transmission). However, these products have not circumvented all of 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 bleed, frequency of bleeding, and goal of treatment for the participant. Both 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; a recently licensed modified FVIII agent with 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 the disease 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). The introduction of rFVIII concentrates in the early 1990's facilitated the use of scheduled protein replacement to prevent bleeding (prophylaxis treatment) as the standard of care for hemophilia rather than on-demand treatment. Prophylaxis 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 as demonstrated in the Swedish prophylaxis studies summarized in Löfqvist, 1997; Ljung, 1998; and Nilsson, 1992.

Prophylactic therapy has revolutionized health outcomes in hemophilia by enabling affected individuals to participate in physical activities and natural history data suggested FVIII:C around 12% of normal may be sufficient to protect from spontaneous joint bleeds (Madhi, 2015; den Uijl, 2011). However, regular replacement therapy poses significant challenges for the hemophilia community, including the frequency of IV infusions required, the 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.

Prophylaxis, which aims to convert the severe phenotype to moderate through regular infusions of clotting factor, with 100% adherence in dosing regimen is more effective than on-demand at preserving joint health (Manco-Johnson 2007; Gringeri, 2011) and has enabled affected individuals to participate more extensively in physical activity (Wang, 2016; Negrier, 2013). Prophylactic therapy for hemophilia has gradually increased among the adult population in the U.S.A. but is still not universally practiced for several reasons. As of 2014, an estimated 85% of children and 63% of adults with hemophilia were on prophylactic regimens (World Federation Hemophilia (WFH) Annual Global Survey 2015). 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, respectively (Croteau, 2015). Furthermore, the

burden of treatment on patients is great. Prophylaxis with either regular or EHL rFVIII protein (half-lives ranges 10.8 -19.7 hours) typically requires 2-3.5 infusions per week (National Hemophilia Foundation, 2016). In a 2001 survey of 38 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 number 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) as an outcome reported a decreased HR-QoL compared with the general population and a positive effect of 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 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 (3.1kb) and exon 26 (1.9 kb), which primarily consists 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 von Willebrand factor (VWF) binding site (Foster, 1988), and the two 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, 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 the 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 (~4.7kb). However, since the B-domain is not required for FVIII activity (Toole, 1986), the CCI [redacted] which produces a B-domain-deleted, but active FVIII protein. Many B-domain-deleted FVIII variants have been constructed, but the derivative the Sponsor has chosen to produce from an rAAV vector is called FVIII-SQ (Lind, 1995). The FVIII-SQ gene produces a single chain translation product, which is cleaved efficiently into an A1-A2 heavy chain and an A3-C1-C2 light chain. This protein is a commercially available product, under the name Xyntha®, and has been used successfully to control and prevent bleeding episodes in individuals with hemophilia A. Thus, expression of FVIII-SQ from a rAAV vector is expected to have the same safety and efficacy profile as Xyntha®.

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, 1984). 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, CCI [redacted] is being used, and transduction using this serotype leads to high levels of FVIII expression and secretion.

2.1.4.2 Biology of Adeno-Associated Virus (AAV) Vectors

Adeno-associated virus 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 is 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 of 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 by the 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 (Pañeda, 2009) 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

Gene therapy has been the goal for curative treatment of hemophilia since the initial cloning of the genes more than 30 years ago. 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, sponsored by St. Jude Children's Research Institute-University College London, demonstrated 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. The first advantage is that precise regulation of transgene expression is not required. The therapeutic range is remarkably wide, from > 1% to 150% of normal. It is clear, based on data from administration of FVIII products into patients with hemophilia A, that levels ≤150% are not associated with ill-effects since the protein circulates as a zymogen (inactive precursor). Second, as stated, > 1% circulating FVIII activity levels may provide protection against chronic arthropathy and CNS bleeding. Patients with levels of > 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). A third advantage of hemophilia A is the availability of large and small animal models of the human disease. Clearly, animal models are major assets in efforts to establish an experimental basis for gene therapy. In the case of hemophilia, 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). Finally, it should be noted that 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

2.2.1 Description of SPK-8011

SPK-8011 (also known as AAV-Spark200-BDD-hFVIII) is a rAAV vector composed of a bio-engineered rAAV capsid (AAV-Spark200) and a codon-optimized expression cassette encodes the SQ variant of a B-domain-deleted human coagulation factor VIII (BDD-hFVIII) gene (Lind, 1995). 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 a B-domain-deleted FVIII CCI variant, namely FVIII-SQ (Lind, 1995), which directs the synthesis of a protein identical to a commercially available BDD-hFVIII protein, Xyntha®.

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:
   - Utilizes AAV-Spark200, which is a novel bio-engineered capsid derived by CCI (Azuma, 2007)

b) Cassette:
   - Encodes the SQ variant of B-domain deleted hFVIII (Lind, 1995) and is codon-optimized CCI (Levitt, 1989)

CCI

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, the final formulation of SPK-8011 may contain a mixture of empty and full capsid particles.

SPK-8011 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-8011

SPK-8011 contains a bio-engineered rAAV capsid (AAV-Spark200) that has been selected for efficient transduction of human hepatocytes and de-selected for transduction of murine hepatocytes, creating a challenge for safety and efficacy testing in mice. An additional challenge is the generation of antibodies against hFVIII in all immunocompetent animals. Thus, the non-clinical pharmacology and toxicology studies utilized 1 species (NHP) to evaluate the safety of the AAV-Spark200 capsid and immunodeficient mice to evaluate the safety of sustained expression of hFVIII. The murine studies relied on another capsid, rAAV-Spark100, to achieve efficient transduction of mouse hepatocytes with the hFVIII transgene.

A study to evaluate the safety of AAV-Spark200 and short-term expression of hFVIII was conducted in cynomolgus macaques. Three cohorts of animals (n=3) were treated with increasing doses of SPK-8011 ($2x10^{12}$, $6x10^{12}$, and $2x10^{13}$ vg/kg). Animals were monitored for clinical observations, body weights clinical pathology (clinical chemistry, hematology, coagulation, urinalysis) over the course of 60 days. In addition, hFVIII antigen levels, FVIII inhibitory antibodies and D-dimer levels were assessed throughout the study. Average hFVIII antigen levels peaked around Weeks 2-3 with 22.3 ± 6.2% hFVIII seen at the low dose, 61.6 ±15.7% at the mid dose and 153 ± 58.1% at the high dose, demonstrating efficient transduction of NHP hepatocytes with this novel AAV serotype. As predicted, the hFVIII levels began to decline around Week 4 in most animals, which correlated with an increase in anti-hFVIII antibodies. In most animals, the antibodies were inhibitory in nature, with some NHPs having inhibitory antibodies with titers as CCI [redacted] Total FVIII activity levels also decreased in these animals. The generation of anti-FVIII antibodies has also been observed by others following hepatic AAV-hFVIII gene transfer in NHPs (McIntosh, 2013). No evidence of thrombosis, as measured by D-dimer levels, was observed, even when hFVIII levels as high CCI [redacted] of normal were observed.

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

2.2.3 Summary of Clinical Experience with SPK-8011

Study SPK-8011-101 is the first study to administer SPK-8011 in humans; however, rAAV vectors have been administered to humans in more than 100 gene therapy clinical trials for a wide range of indications, including multiple studies in hemophilia A and B (George, 2017; High, 2011; Rangarajan, 2017; Miesbach, 2016; Nathwani, 2014; Nathwani, 2011b; Manno, 2006; Monahan, 2015; Mingozzi, 2011a).

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

- In general, AAV-mediated gene therapy continues to demonstrate an acceptable safety profile.
- 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.
- Neutralizing antibodies against FVIII or FIX have not been reported for any participant to date.
- Liver-directed AAV-mediated gene transfer may result in development of an apparent dose-dependent immune response to the vector capsid, as evidenced by a transient and asymptomatic rise in transaminases (ALT and/or AST) and a decline in factor level This response can potentially lead to immune-mediated clearance of vector-transduced hepatocytes and an eventual loss of transgene expression.
- At the lower doses infused, the transient elevation of transaminases is typically attenuated by a tapering course of corticosteroid therapy to prevent loss of AAV-transduced hepatocytes. At higher doses, some participants have required additional immunomodulation to control the immune response during the corticosteroid taper; other participants have lost all expression despite initiation of reactive steroids at the first sign of elevation or upward trend of ALT with or without coincident unexpected decline in FVIII expression.
- Early initiation of prophylactic corticosteroids in the higher dose ($2x10^{12}$ vg/kg) resulted in a transient supraphysiologic increase in local FVIII levels to greater than 200%. An intermediate dose cohort ($1.5x10^{12}$ vg/kg) was therefore initiated. The mechanism of action for the FVIII increase has been examined in a nonclinical study in mice where the administration of prednisolone in combination with SPK-8005 (which contain the same transgene and regulatory elements as SPK-8011 encapsidated in a capsid CCI [redacted]) transiently increased mean human FVIII expression 1.5- to 3.2-fold compared with animals treated with SPK-8005 alone.
- The attempt to use oral corticosteroids CCI [redacted], though successful in achieving long-term expression of FVIII associated with hemostatic control, also resulted in the need for prolonged courses of corticosteroids, resulting in undesirable steroid-associated side effects. In some cases, it was difficult to

taper off steroids except by adding another immune suppressive agent. For this reason, prophylactic steroids will no longer be pursued.

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 below, and input from clinical experts in rheumatology, organ transplantation, viral immunology, and bone marrow transplantation, the 2 regimens shown in Figure 1 will be evaluated. These represent 2 distinct approaches to the problem of a $CD8^+$ T cell response to the AAV capsid. CCI [redacted]

2.2.4 Summary of Non-clinical Experience with Tocilizumab

In order to test whether blockade of IL-6 receptor signaling with the humanized monoclonal antibody, TCZ (Actemra®) could decrease immune response in the context of AAV-mediated gene delivery, 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 IL-6 blockade increasing the percent of animals developing anti-transgene antibodies, as observed with other immunosuppressive drugs (Mingozzi, 2007). However, since virtually all NHP 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, CCI [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 CCI [redacted] animals were divided into 2 experimental groups (n=5/group). One of the groups received an IV CCI [redacted] 1 day prior to vector infusion. CCI [redacted] The next day, both groups received an IV infusion of CCI [redacted].

No animals demonstrated hypersensitivity or anaphylactic reactions following administration of either TCZ or CCI [redacted]. Animals were bled every week for analysis of safety endpoints and hFIX transgene expression levels, along with monitoring of circulating IL-6 levels. 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 TCZ 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 CCI vector alone or in combination with TCZ. 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 TCZ compared to controls.

IL-6, 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. CCI Importantly, TCZ administration was well tolerated and did not seem to affect hepatic transduction. CCI In control animals, associated IgG and neutralizing antibody titers against the capsid developed at markedly elevated levels and persisted through the duration of the study. CCI

In regards 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). Peripheral blood mononuclear cells (PBMC) from control animals and the TCZ group were isolated and subsequently re-stimulated in vitro with the CCI vector. At all timepoints (Days 28-84), the mean number of PBMCs secreting TNFα were lower in the TCZ-treated animals compared to controls. The observations in PBMCs were correlated with those from livers which showed that TCZ treatment promoted a long-lasting anti-inflammatory milieu in the liver. Increased secretion of the anti-inflammatory cytokine IL-10 was observed in mixed liver cell culture from TCZ-treated animals, but was not measurable in cultures from animals that received CCI vector alone. Further, immunophenotyping of cell surface major histocompatibility complex (MHC) class II expression on liver sinusoidal endothelial cells from TCZ-treated animals was consistent with expression of a more tolerogenic phenotype.

These preclinical studies indicating the potential for IL-6 receptor blockade to modulate AAV vector immunogenicity are of interest in light of observations in a trial of AAV vector mediated gene transfer for hemophilia B, in which initial transgene FIX expression was achieved and then lost in all participants except for 1, who instead has sustained FIX correction for > 3 years. Whole exome sequencing of all participants demonstrated that the single participant who avoided apparent immune-mediated loss of transgene expression carried a functional polymorphism in the IL-6 Receptor gene, predicted to result in diminished IL-6-mediated pro-inflammatory signaling (Bilic, 2019). Altogether, these data support the use of TCZ in participants receiving AAV-based gene therapy treatment as a safe and potentially effective strategy to modulate vector immunogenicity.

2.2.5 Summary of Non-Clinical and Clinical Experience with Mycophenolate Mofetil

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. The immunomodulation regimen included tacrolimus (FK506) and MMF. The hFIX transgene expression cassette used in this study was the same as initially used in IND 9398 and contained the wild type copy of the hFIX cDNA.

Results have been published (Jiang, 2006) demonstrating that administration of AAV8-hAAT-hFIX16 was safe and effective in 5 out of 6 monkeys at a dose of $5x10^{12}$ vg/kg, provided there were low or undetectable levels of pre-existing AAV8 neutralizing antibody (NAb). Co-administration of MMF and FK506 with AAV8 vectors was safe and did not alter the efficiency of liver transduction in NHPs.

The regimen of FK506 and MMF has also been used successfully in treating patients who undergo liver transplantation to prevent graft rejection (Post, 2005; Perry, 2005). MMF has been administered along with rapamycin to a single participant who received AAV2-hFIX16 under IND 9398. The side effect profile was acceptable and there were no serious adverse events (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). Long-term follow-up data are not available on another participant who received a 16-week course of MMF and rapamycin; this participant died approximately 2.5 years later of bleeding complications related to hemophilia.

MMF has been used to treat individuals with congenital hemophilia A and hemophilia B as well as acquired hemophilia A for periods of months as part of combined immune suppression approaches for the eradication of inhibitor antibodies. In these case series, infections have been the most common complication with incidence similar to that described with the use of MMF in rheumatologic conditions. Transient neutropenia has also been reported, although attribution to MMF when used in combined drug regimens is not clear from these reports. No transaminase elevations and no exacerbation of underlying hemophilic bleeding are reported in these series.

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 will be monitored for 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

As of 24 November 2020, there has been 1 report of symptoms associated with SPK-8011 (rAAV-FVIII), occurring in the evening following SPK-8011 vector infusion at the dose of $2x10^{12}$ vg/kg.

Vomiting, pyrexia, low 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). Allergic-type reactions, including anaphylaxis, have been reported for all rFVIII protein products. These events are rare and have occurred in close temporal relation with the development of FVIII inhibitors. 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

Inhibitor development most often occurs within the first 20 infusions of FVIII replacement therapies. There is a possibility that participants infused with SPK-8011 will develop inhibitors to FVIII, although inhibitor development has not occurred in any of the participants who have received AAV2-hFIX, AAV8-hFIX (Nathwani, 2014), AAV8-hFIX19 (under investigational new drug application (IND) 15149), BAX-335 (under National Clinical Trial (NCT)# 01687608). In addition, as of 24 November 2020, inhibitor development has not been reported in any of the 17 participants in this study. Moreover, no inhibitors have been reported in the recent BioMarin trial of an AAV5-BDD-hFVIII (BMN-270) (Rangarajan, 2017; Pasi, 2020) nor in ongoing Study SPK-8016-101 as of 26 October 2020. The likelihood of participants in this study developing inhibitors will be minimized by including only individuals who do not make inhibitor antibody to FVIII, despite prior heavy exposure to the FVIII infused protein (i.e., those who have had greater than 150 EDs to FVIII concentrates). 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

As of 24 November 2020, of the 17 enrolled participants, 5 of them (29%) experienced treatment-emergent adverse events (TEAEs) reported as transaminases or alanine transaminase (ALT) increase, 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. The cause of a transient and asymptomatic hepatic transaminase elevation (i.e., elevated alanine aminotransferase and/or aspartate aminotransferase) 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). Transaminase elevation (progressive elevation of hepatic transaminases of approximately 1.5- to 2.0-fold above screening/baseline accompanied with the loss of transgenic FVIII activity levels should it occur, will be managed in this study with immunosuppressive therapies in tapering dose schedules. In the setting of AAV-FIX, corticosteroids have generally been effective for the hepatic transaminase elevations observed in the context of AAV vector infusion to the liver (Nathwani, 2014); at the higher doses required for AAV-FVIII, this has not always been the case.

2.3.1.4 Anti-AAV Neutralizing Antibody Development

As is expected after systemic administration of AAV vectors, all 17 participants (100%) treated with SPK-8011 developed neutralizing antibodies to the AAV capsid. This risk seems to have no immediate impact on the expression of FVIII from 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 doses levels of SPK-8011 may not be sufficient to raise the vector-derived FVIII:C levels to a therapeutic level for control and prevention of bleeding episodes when the participants halt their prophylaxis (factor or non-factor replacement) treatment after vector administration. Clinical experience suggests most patients can stop routine prophylaxis within 2 weeks of receiving SPK-8011. 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 (Appendix 13.1).

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 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 CCI of intravenously dosed methylprednisolone (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. While on corticosteroids, participants will also be monitored for side effects including opportunistic infections.

Corticosteroids such as methylprednisolone, 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.

As of 24 November 2020, of the 17 enrolled participants in Study SPK-8011-101, 3 of them (Participants 5, 15, and 16) experienced adverse events (AEs) presumed to be related to immunomodulatory therapy (e.g., adrenal insufficiency, gastroesophageal reflux disease,

osteoporosis, irritability, weight gain, generalized edema, acne, hypomagnesemia, hot flashes, and hyperactivity). In addition, as of 26 October 2020, steroid-associated and nonserious AEs (e.g., cushingoid appearance, candida infection, swelling face, tooth infection, increased blood glucose, and fatigue) have been reported due to excessive long-term use of corticosteroids in 3 of the 4 participants in a similar hemophilia A gene therapy study (SPK-8016-101) initiated by the Sponsor.

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).

The planned prophylactic regimens of TCZ and MMF 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 all occur with greater 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 “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 for the dosing of oral azathioprine in addition to prednisone/prednisolone therapy to provide therapeutic benefit while using lower steroid doses and/or to achieve successful taper off corticosteroids. Additional immune modulating agents, including tacrolimus and MMF, have been used to overcome suboptimal response to corticosteroid monotherapy or corticosteroid/azathioprine combined therapy in the setting of immune-mediated hepatic inflammation. Participants who are treated with azathioprine, tacrolimus, or other immune suppressive agents will be monitored closely as described above (Section 2.3.1.6) for side effects and to allow 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-8011-101 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 planned day of administration of SPK-8011. If a participant is currently receiving immunosuppressive medication, timing of vaccinations could be adjusted around the participant's course of immune suppressant treatment.

2.3.1.7.1 Azathioprine

Azathioprine is an immunosuppressive purine antimetabolite. Side effects from azathioprine may include cytopenias (in particular, bone marrow suppression leading to neutropenia and thrombocytopenia), nausea, vomiting, diarrhea, rash, fever, and arthralgia. Hepatotoxicity, presenting as liver transaminase elevations (may be idiopathic or associated with cholestatic hepatitis) with or without abdominal complaints, is uncommonly observed, however the risk is increased in association with chronic liver disease.

As of 24 November 2020, in this ongoing SPK-8011-101 gene therapy study, 1 participant was prescribed azathioprine, used to aid in the taper from prednisone, CCI [redacted]. As of 26 October 2020, in a similar hemophilia A gene therapy study (SPK-8016-101) initiated by the Sponsor, 2 participants received azathioprine during the taper off corticosteroids. One participant experienced azathioprine-induced nonserious hepatotoxicity which was characterized by transient increases of alanine aminotransferase (ALT) (2.3 x ULN), aspartate aminotransferase (AST) (2 x ULN), gamma-glutamyl transferase (GGT) (6 x ULN), and lactate dehydrogenase (LDH) (1.2 x ULN), occurring within 1 week of escalating from a dose of CCI [redacted]. Enzyme levels returned to normal 10 days after azathioprine was stopped. A second individual on the SPK-8016-101 trial was prescribed CCI [redacted] as an aid to taper and discontinue prednisone, without transaminitis or other adverse effects.

Populations at special risk for side effects from azathioprine therapy include patients with very low thiopurine methyltransferase (TPMT) activity. Patients with near-zero erythrocyte concentrations of TPMT activity are at risk for myelosuppression during azathioprine treatment (Manns, 2010). Only 0.3%-0.5% of the population has a severe enzyme deficiency, and not all patients with a deficiency of this degree experience bone marrow failure. Individuals will be tested at screening for SPK-8011 gene therapy for TPMT deficiency prior to initiation of azathioprine dosing. Chronic immunosuppression with azathioprine increases the risk of malignancy so azathioprine should be used with caution in individuals with malignancy.

2.3.1.7.2 Tacrolimus

Tacrolimus is an immunosuppressive calcineurin inhibitor that interferes with several calcium-dependent processes in immune cells. The most common adverse reactions to tacrolimus include abnormal renal function, hypertension, diabetes mellitus, fever, tremor, paresthesias, hyperglycemia, cytopenia (leukopenia, anemia), abdominal complaints, electrolyte abnormalities (hyperkalemia, hypomagnesemia) and hyperlipemia. More severe neurotoxicity is described at higher whole blood trough concentrations and therapeutic drug monitoring is required in the trial. As with other immune suppressive agents, chronic use is associated with increased risk of infection

and malignancy, in particular lymphoma and skin cancer. Tacrolimus should be used with supervision by a physician with experience in immunosuppressive therapy. Tacrolimus may induce changes in blood concentrations of, and also be affected by use of, concomitant medications, and potential drug-drug interactions with tacrolimus must be considered during therapy (Manns, 2010).

As of 24 November 2020, in this ongoing SPK-8011-101 gene therapy study, 2 participants have been prescribed tacrolimus, used to aid in the taper from prednisone. CCI [redacted]. One participant was prescribed tacrolimus CCI [redacted] during the prednisolone taper. The tacrolimus dose required frequent monitoring and adjustment due to apparent pharmacokinetic interactions between tacrolimus and the participant's concomitant use of highly active anti-retroviral therapy (HAART). The addition of tacrolimus allowed wean from all immune suppressive therapy without recurrence of hepatic inflammation and the participant sustains a current FVIII level of 15%. The immunosuppressive taper of a second participant used co-therapy with prednisone and tacrolimus CCI [redacted]. Mild elevations of the serum creatinine have been observed in the local laboratory and have corrected with adjustment of the tacrolimus dose. During the prednisone and tacrolimus taper, the FVIII level declined and MMF was added to the immunomodulation regimen. MMF and tacrolimus were used concomitantly CCI [redacted] without AE.

2.3.1.7.3 Tocilizumab

TCZ is a humanized monoclonal IgG antibody that binds to and interferes with the function of both soluble and membrane-bound interleukin-6 receptors. The following side effects have been reported in patients taking TCZ: upper respiratory tract infection, nasopharyngitis, headache, hypertension, dizziness, bronchitis, rash, mouth ulceration, abdominal pain, gastritis, transaminase increased, circulating neutrophil count decreased, and hypercholesterolemia. Side effects associated with a single dose or short courses of TCZ include hypersensitivity/anaphylaxis, neutropenia, thrombocytopenia, hypofibrinogenemia, and hepatotoxicity including transaminase elevations. Increases in the transaminases have been reported days to months following an infusion of TCZ (National Institute of Diabetes and Digestive and Kidney Diseases, 2015). Rare cases of liver failure have been ascribed to TCZ, however these have occurred with chronic administration in the setting of rheumatoid arthritis. The effect on ALT of 1 or 2 doses of IV TCZ has been reported in adults with rheumatoid arthritis (Emery, 2019). A median increase of 10-15 U/L was observed within 2-4 weeks of 1-2 doses of 4 mg/kg or 8 mg/kg IV TCZ. Elevations to > 3 x ULN were demonstrated in 0 out of 25 individuals who received a single dose of 4 mg/kg IV and 2 out of 24 participants who received a single dose of 8 mg/kg IV. Of note, these study participants were also treated concomitantly with methotrexate, a potentially hepatotoxic drug. Transaminitis generally resolved within weeks of discontinuing TCZ dosing.

In this trial of SPK-8011, individuals in cohort 2 will receive TCZ. Elevations of ALT that occur within CCI [redacted] following gene delivery, if seen, will likely result from TCZ exposure. Elevations of ALT that occur later than 6 weeks following gene therapy CCI [redacted]

CCI [redacted] will be possibly or probably the result of cellular immune response directed against AAV-transduced hepatocyte, in particular if observed with coincident decrease in the circulating transgenic FVIII activity.

Anaphylaxis that required treatment discontinuation has been reported in 0.1-0.9% of patients using repeated doses of TCZ for the treatment of a variety of autoimmune diseases (Actemra® Prescribing Information, 2020). Anaphylaxis most commonly does not occur with the first exposure but instead with the second to fourth exposure to TCZ. Neutropenia has been observed following treatment with TCZ. Most typically, absolute neutrophil counts decreased to the nadir 3 to 5 days following TCZ administration and thereafter, neutrophils recovered towards baseline in a dose dependent manner. Thrombocytopenia has been observed following treatment with TCZ. Elevations in lipid parameters (total cholesterol, LDL, HDL, triglycerides) have been reported at 6 weeks following initiation of TCZ. Elevated lipids may be managed with lipid lowering agents. Clinically, participants will be monitored closely for neutropenia and evidence of opportunistic infections while they are on TCZ. Serious infections leading to hospitalization or death including tuberculosis (TB), bacterial, invasive fungal, viral, and other opportunistic infections have occurred in patients receiving TCZ. The most common serious adverse reactions associated with long-term repeated dosing of TCZ are infections, including serious and opportunistic infections, with the incidence of infection associated with the length of the course of TCZ. With respect to this point, it should be noted that the contemplated course of TCZ therapy in this protocol is much shorter (from 1 to 2 doses) than the regimens used in more typical indications, requiring long term immunomodulation. In the setting of prolonged administration, activation of latent tuberculosis has been observed. The incidence of tuberculosis in worldwide clinical development programs is 0.1%. Participants will be screened for latent tuberculosis and excluded from the TCZ arm if latent or active tuberculosis is observed. Cases of herpes zoster exacerbation have been reported. The impact of treatment with TCZ on the development of malignancies is not known but malignancies were observed in clinical studies. TCZ is an immunosuppressant, and treatment with immunosuppressants may result in an increased risk of malignancies. For a detailed description of the risks associated with TCZ, please see Actemra® US Prescribing Information (2020).

2.3.1.7.4 Mycophenolate Mofetil

Mycophenolate mofetil is an antimetabolite immunosuppressant. The following AEs have occurred in patients receiving MMF: hypertension, edema, low blood cell counts, pain, diarrhea, nausea, vomiting, increased cholesterol and increased risk of infection. Clinically, participants will be monitored closely for neutropenia and evidence of opportunistic infections while they are on MMF. Investigators will be sensitive to gastrointestinal complaints from the participants while they are on MMF.

Patients receiving immunosuppressive regimens involving combination drugs, including MMF, are at increased risk of developing lymphomas and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving CCI [redacted] in controlled clinical trials of renal, cardiac, and hepatic transplant patients (CellCept US Prescribing Information,

2019). Again, it should be noted that these findings were seen in individuals receiving long-term treatment with MMF.

Cases of progressive multifocal leukoencephalopathy (PML), sometimes fatal, have been reported in patients treated with MMF. Hemiparesis, apathy, confusion, cognitive deficiencies and ataxia were the most frequent clinical features observed. The reported cases generally had risk factors for PML, including treatment with additional immunosuppressant therapies and impairment of immune function. MMF is usually taken for many years to prevent organ rejection after heart, liver, or kidney transplantation. However, the duration of MMF use in the context of this study will be much shorter (weeks to months), which mitigates the risk of developing serious side effects. For a detailed description of the risks associated with MMF, please see CellCept US Prescribing Information (2019).

The risk of genotoxic effects on sperm cells cannot be excluded with the use of MMF. Thus, sexually active male patients and/or their female partners are recommended to use effective contraception during treatment of the male patient and for at least 90 days after cessation of treatment.

As of 24 November 2020, in this gene therapy Study SPK-8011-101, 2 participants have been prescribed MMF to control an apparent immune response not controlled with prednisone alone. One participant was prescribed MMF after the concomitant use of tacrolimus and prednisone did not fully suppress an immune response. MMF was added CCI [redacted], and MMF and tacrolimus were concomitantly administered for CCI [redacted]. The participant remains on MMF as a stand-alone immunomodulator. The other participant completed an immunosuppressive taper using co-therapy with prednisone and MMF. MMF has been administered for CCI [redacted] with no AEs associated with MMF. The addition of MMF allowed weaning from prednisone without recurrence of hepatic inflammation and the participant currently has a FVIII level of 4.0%.

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

Immune suppressive medications including corticosteroids, TCZ, MMF, azathioprine and tacrolimus, 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 of 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 TCZ 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 additional clinical evidence accrues, it is advised to consider participants using immune suppressive medication in the SPK-8011 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-8011-101 clinical trial participants should consider individualized approaches to social/behavioral interactions as well as clinical visits and treatment approaches consistent with national, local and institutional COVID-19 prevention guidance (U.S. Food and Drug Administration, 2020).

2.3.2 Potential Benefit

The main purpose of this study is to evaluate the safety of SPK-8011 at up to 3 or 4 different dose levels. It is not known if the dose levels will raise the activity levels of FVIII in humans, though based on prior clinical experience in hemophilia B and non-clinical experience in NHPs with SPK-8011, it is hypothesized that the dose levels tested may result in detectable FVIII:C. Current commercially available FVIII concentrates exhibit in vivo half-life (ranging from 10.8 to 19.7 hours) which require IV injections of approximately every 2 to 3.5 days for an effective prophylactic treatment (National Hemophilia Foundation, 2016). SPK-8011 has the potential to reduce the frequency of administration in the prevention of bleeding episodes. Additionally, important scientific insights about rAAV-mediated gene transfer for hemophilia are expected from the data generated in this proposed study.

2.4 Study Rationale

Published data from Nathwani and colleagues (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 levels (±SD) 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 out of these 6 participants developed an asymptomatic rise in hepatic transaminases between Weeks 7 and 9 after vector administration, which required a short, approximately 8-week tapering course of prednisone therapy (Nathwani, 2014) to prevent loss of FIX transgene expression. The recent results from an AAV5-mediated B-domain-deleted human factor VIII (AAV5-BDD-hFVIII; BMN-270) gene transfer trial for severe hemophilia A resulted in sub-therapeutic levels (0.6% FVIII activity levels) over 34 weeks in 1 participant at $6x10^{12}$ vg/kg (low dose-level), transgene FVIII activity levels 2.3% of normal over 26 weeks in 1 participant at $2x10^{13}$ vg/kg (middle dose-level), and lastly, FVIII activity levels 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 show FVIII within the normal range, and 1 out of 7 in the range of 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 of 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 the vector administration. These 6 participants given prophylactic corticosteroids had exposure from 15 weeks to at least 35 weeks in 1 participant (Pasi, 2020).

The Sponsor conducted a clinical study of a novel bio-engineered rAAV vector encoding the high-specific activity FIX variant vector (AAV-Spark100-hFIX39-Padua or SPK-9001) for hemophilia B (IND 16437). The first 10 participants who received the initial dose level of $5x10^{11}$ vg/kg, achieved endogenous FIX activity levels ranging from 14-81% (High, 2016; George, 2017). Steady-state FIX expression was reached by 12 weeks after the infusion of SPK-9001, resulting in a mean FIX activity level of 33% of normal. Natural history data of patients with hemophilia suggests that circulating factor levels of approximately 12% of normal are required to protect against spontaneous joint bleeds (den Uijl, 2011). All participants dosed with SPK-9001 have discontinued routine infusions of FIX concentrates. One participant with severe joint disease self-administered precautionary infusions for knee pain. In the study to date, no SAEs have been reported, including no factor IX inhibitors and no thrombotic events. Two of the 10 participants experienced an asymptomatic, transient elevation in liver enzymes, or decline in FIX activity, potentially indicative of an immune response to the vector capsid, that occurred several weeks post infusion. Both participants received a tapering dose of oral corticosteroids, after which their alanine aminotransferase (ALT) levels returned to baseline

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

The objective of this study is to determine the safety, tolerability, and transgene-derived FVIII activity levels of a single IV infusion of SPK-8011 (AAV-Spark200-BDD-hFVIII) in individuals with hemophilia A.

2.5 Rationale for Dose and Schedule Selection

Planned dose levels include $5x10^{11}$, $1x10^{12}$, $2x10^{12}$, and $1.5x10^{12}$ vg/kg of SPK-8011.

At the proposed starting clinical dose of $5x10^{11}$ vg/kg, FVIII activity levels around 3% of normal are hypothesized to be achieved, based on studies in cynomolgus macaques (see Section 2.2.2). While a universal preclinical model to determine AAV dosage in humans does not exist, Spark's previous experience in NHPs using AAV2, AAV8 and AAV-Spark100 vectors to mediate liver-derived expression of coagulation factor IX indicates that macaque models have been a good predictor of vector efficacy in humans. More recently, chimeric "humanized" mice with livers partially repopulated with human hepatocytes have become a valuable tool to determine hepatic transduction efficacy of different viral capsids. CCI [redacted]

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 are unlikely to qualify for future gene transfer clinical studies. For this reason, the Sponsor proposes the clinical starting dose level of $5x10^{11}$ vg/kg, which may result in a reasonable therapeutic level, but also provides an ample safety margin for the participants.

Safety of these dose levels is supported by previous and ongoing human clinical trials of AAV2, AAV5, AAV8, AAVrh10 and AAV-Spark100 vectors encoding hFIX or hFVIII, 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. This dose level is ~10-fold higher than the proposed starting clinical dose level of $5 x10^{11}$ 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). For this reason, the final formulation of SPK-8011 may contain a mixture of empty and full capsid particles.

3 OBJECTIVES AND ENDPOINTS

Objectives
Primary
• To evaluate the safety and tolerability of SPK-8011. • To evaluate the efficacy of SPK-8011.
Secondary
• To determine the PK characteristics of SPK-8011. • To characterize the immune response to the vector and transgene product.
Endpoints
Primary
• For safety and tolerability: o Clinically notable changes from baseline in physical examinations and vital signs. o Incidence of adverse events, including clinically significant abnormal laboratory values. o Hepatic transaminase elevation requiring immunosuppression. • For efficacy: o Primary kinetic parameters of peak and steady-state FVIII activity levels assessed by coagulation clotting assays. o Number of FVIII infusions after vector administration. o Number of bleeding events (spontaneous and traumatic) after vector administration.
Secondary
• Additional kinetic assessments will include, but not limited to: o Time to achieve steady-state FVIII activity level; o Vector-shedding of SPK-8011-101 in bodily fluids. • Incidence of immune responses to AAV capsid protein and BDD-hFVIII transgene.
Exploratory
• Joint assessments: o Number of target joints o Hemophilia Joint Health score • Activities assessments: o Hemophilia Activities List o Change in Level of Activity questionnaire • Quality-of-life assessments: o Haem-A-QoL questionnaire o Euro Quality of Life Five Dimensions Questionnaire (EQ-5D-5L) questionnaire • Health-economic parameters to include, 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

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

4 STUDY DESIGN

4.1 Overall Design

This 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-8011 in men with severe hemophilia A. Up to 50 eligible participants will be dosed with a single IV infusion of SPK-8011.

4.1.1 Sequence of Enrollment

The following 2 staggering strategies are employed 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* (U.S. Food and Drug Administration, 2015):

- The first 2 participants in each dose-level ($5x10^{11}$ vg/kg, $1x10^{12}$, $2x10^{12}$ vg/kg) will be infused with SPK-8011 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 up to 4 participants who have received SPK-8011 at a given dose level prior to dosing the first participant in the next dose level.

The $1.5x10^{12}$ dose arm was initially evaluated in 3 participants using prophylactic corticosteroids (Amendment 4) and 1 participant using reactive corticosteroids (Amendment 5). This dose will be further evaluated using 2 cohorts to explore immunomodulation. Cohort 1 will utilize CCI [redacted]. Reactive corticosteroids will be permitted in Cohorts 1 and 2, if indicated, in response to clinical observations consistent with an immune response that occurs despite TCZ or MMF. Use of azathioprine, MMF, and/or tacrolimus will be permitted in any participant 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.

CCI [redacted]

CCI [redacted]

Reactive corticosteroids will be permitted in Cohort 2, if indicated, in response to clinical observations consistent with an immune response that occurs despite TCZ. Use of azathioprine, MMF, and/or tacrolimus will be permitted in any participant 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.

Cohort	Number of Participants	SPK-8011 Dose
1	3-5	$1.5x10^{12}$ vg/kg
2	3-5	$1.5x10^{12}$ vg/kg

CCI [redacted]

*CCI [redacted]

Continued enrollment will alternate between Cohorts 1 and 2 of $1.5x10^{12}$ vg/kg. A stagger of 6 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).

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 cohorts. For Cohorts 1 and 2 of the $1.5x10^{12}$ dose, the first 2 participants will be infused at least 6 weeks apart. If either of the immunomodulatory regimens are successful and if agreed to by the DMC, a third Cohort will be introduced using that same CCI [redacted] SPK-8011.

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:

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

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.

It is expected that corticosteroid therapy may increase the transgene expression from the SPK-8011 expression cassette, as observed in both animal models and in our clinical observations using both early prophylactic and the reactive application of corticosteroids. 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 participant remains on corticosteroid treatment. Instead, expression level of steady-state FVIII:C as an efficacy endpoint is evaluated after daily oral corticosteroids or CCI steroids have been discontinued for at least 2 weeks. Note that were the concomitant use of steroids to have an effect to artifactually increase 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-8011 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. Nevertheless, at the time of the current protocol version, the SPK-8011 vector has already been observed in this clinical trial following escalation across the entire range from $5x10^{11}$ vg/kg through $2x10^{12}$ vg/kg of SPK-8011. No participant has achieved the ceiling expression level of steady-state FVIII:C > 80% of normal that is detailed above.

Tocilizumab is expected not to influence levels of the expression of the transgene product, as evaluated in pre-clinical animal studies (summarized in Section 2.2.4). Tocilizumab is also not expected to influence or confound the estimation of the treatment effect of the vector to correct deficient levels of FVIII.

MMF is expected not to influence or confound the estimation of the treatment effect of the vector to correct deficient levels of FVIII. MMF was administered in 2 participants in this study, SPK-8011-101, as of 24 November 2020, and did not appear to have an influence on the transgene expression of FVIII.

After escalation through the first 3 planned dose levels, an intermediate dose level (e.g., $1.5x10^{12}$ vg/kg of SPK-8011), was agreed upon 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 cohort ($2x10^{12}$ vg/kg) to better evaluate the safety, efficacy, and variability of response within a given cohort. Any decision to add an intermediate 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 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 (IND 9398, IND 15149, and IND 16437), 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-ɣ ELISpot (Manno, 2006; Mingozzi, 2007; Nathwani, 2011b). 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 one or more of the following triggers: declining FVIII levels, rise in ALT above participant baseline or elevated IFN-γ 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 these individuals eventually returned to hemophilia prophylaxis regimens, one with recombinant FVIII, the other with emicizumab. Based on clinical experience with FIX, there is no evidence that there is significant hepatic toxicity related to these transient elevations, and most appear to resolve spontaneously without symptoms. In the FIX Phase 1/2 study, 3 of 15 participants receiving a dose of $5x10^{11}$ required a short course of oral corticosteroids (George, 2017; George, 2019) due to elevation of the ALT above the patient's baseline. These transient elevations may be accompanied by a decrease in factor activity (Manno, 2006; Nathwani, 2011b). 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. This approach has been used in other trials where AAV has been administered systemically (including hemophilia NCT#02576795; Rangarajan, 2017, and spinal muscular atrophy NCT#02122952; Mendell, 2017). This protocol was amended to incorporate prophylactic steroids, beginning 4 weeks after vector infusion and continuing in a tapering regimen until at least 12 weeks post vector infusion (Protocol Version 4). In the first 2 participants administered SPK-8011 at $2x10^{12}$ vg/kg using prophylactic oral corticosteroids (CCI [redacted]), 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 these participants, early initiation of corticosteroids resulted in a transient supraphysiologic increase in FVIII levels to greater than 200%.

Based on discussions with the study's independent Data Monitoring Committee, 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. In 2 individuals, cessation of corticosteroids (initiated early, at approximately 4 weeks) was followed by a return of positive IFN-γ ELISpots against capsid, decline in FVIII levels, and increases in ALT/AST. Restarting corticosteroids returned transaminases to baseline levels and stabilized FVIII levels. In some cases, it has been clinically necessary to initiate a steroid sparing regimen(s) (e.g., azathioprine, tacrolimus) to aid in the prednisone taper. In these participants, a steroid sparing agent has allowed a taper of the prednisone dose to CCI [redacted]. Because of the need for 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.

At the lower doses of SPK-8011 which demonstrated durable FVIII expression associated with hemostatic protection, transient elevations of hepatic transaminases have been adequately attenuated using a tapering course of oral corticosteroids, consistent with the experience in liver-directed AAV gene therapy trials for hemophilia B. At the highest dose of SPK-8011 tested ($2x10^{12}$ vg/kg) reliance upon reactive or early prophylactic tapering courses of daily oral

corticosteroids have not yielded satisfactory outcomes. Although the FVIII activity levels returned to the normal range in both participants administered $2x10^{12}$ vg/kg as the prophylactic oral corticosteroid dose was tapered, the transient supraphysiologic levels represented a potential safety concern. Reactive oral corticosteroids, initiated promptly, did not prevent complete loss of transgenic FVIII expression in 2 participants. CCI , given in an attempt to prevent CCI , was associated with the observation of delayed and prolonged signals of hepatic inflammation, greatly extended prednisone exposure, and corticosteroid-associated side effects. Specifically, for the purpose of maintaining immune quiescence while completing corticosteroid taper, three additional immune modulating agents (tacrolimus, MMF, and azathioprine) have been used.

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, using IV CCI steroids as the preferred method. Oral corticosteroids may be utilized, if clinically indicated. ELISpot is an exploratory assay and not used as a clinical predictor for immunomodulation.

The reactive approach using IV corticosteroids seeks to potentially optimize the reactive use of corticosteroids when initiated in response to clinical signals of transduced hepatocyte loss. This approach explores IV CCI methylprednisolone as an alternative to daily tapering oral prednisone. Experience with the use of IV CCI corticosteroids in a variety of autoimmune disorders, including during organ-threatening disease flares, suggests efficacy with potentially limited exposure (when compared to tapering daily oral regimens). Supportive care with the additional immunomodulators tacrolimus, MMF, or azathioprine may be employed to spare cumulative steroid exposure, and the possible side effects of these potential supportive adjuncts are reviewed.

In light of the complications observed with prolonged exposure to corticosteroids observed in 4 participants in Study SPK-8011-101, Cohorts 1 and 2 will be investigated to prevent the initiation of the CCI , evaluated in a separate study group. Cohorts 1 and 2 will receive MMF and TCZ, respectively, CCI . Prophylactic corticosteroids will not be explored; should apparent immune mediated hepatocyte loss be observed, support with reactive corticosteroids may be initiated.

Significant elevations of transaminases (≥ 5-10 x 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 CCI or tapering regimen, IV and/or oral, until clinical concern of hepatic inflammation is resolved and per investigator/Sponsor discretion.

4.1.3.1 Corticosteroid Regimen

If transaminases become elevated or the FVIII declines, IV corticosteroids (methylprednisolone at CCI for 3 consecutive days and repeated 1 week following the CCI is recommended in consultation with the Sponsor. CCI IV corticosteroids may be followed by a maintenance daily dose of approximately CCI or until the next course of CCI corticosteroids. The CCI steroid interval should taper when the transaminases and FVIII have stabilized.

IV CCI corticosteroids are the preferred approach. The suggested regimen is IV methylprednisolone CCI and repeated 1 week following the CCI if immune triggers are not resolved. A remote provider or home nursing service may be utilized for the administration of IV corticosteroids. In these instances, storage of IV medication and supplies may be required in the home. Local policies and procedures for home storage and nursing care should be followed.

Additional weekly CCI steroid courses may be repeated in response to a trigger from the transaminases or FVIII level. The CCI course should reduce in frequency to CCI 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 IV CCI 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 (IV CCI 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-CoV2 (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 or corticosteroids may at the discretion of the investigator be delayed 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-CoV2 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 AEs. In the AASLD and the EASL guidelines for the treatment of autoimmune hepatitis, an alternative regimen of corticosteroids and steroid sparing agents, azathioprine, MMF, or tacrolimus, 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. Azathioprine, MMF, and tacrolimus have been utilized as steroid-sparing agents in the most recent iteration of this study, to good effect in each case, and are described below.

CCI mg may be considered in participants who are having difficulty tapering off prednisone, however CCI may be necessary to prevent hepatotoxicity. The CCI in consultation with the sponsor. CCI ay also be considered as a primary or second line agent in a steroid sparing regimen.

An alternative option may be considered of using tacrolimus (another immunomodulatory agent that antagonizes activation and proliferation of T cells) CCI to the participants who are not responding to corticosteroids or currently failing the steroid taper, in an effort to reduce or stop the prednisone. Therapeutic drug monitoring will be done twice per week with the aim of CCI . Timing of trough levels may be altered by the Investigator based on clinical response. The tacrolimus dose CCI and the prednisone will be tapered subsequently. CCI

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 steroid-sparing immune suppressive agents.

4.1.3.3 Prophylactic Use of TCZ and MMF

The data from the non-GLP study in NHPs described in Section 2.2.4 provides proof-of-concept that blockade of IL-6 signaling by administration of the humanized monoclonal antibody prior to AAV infusion can reduce both the innate and adaptive immune responses to AAV. In Cohort 2, CCI followed by

evaluation of the data by the Sponsor in consultation with the Data Monitoring Committee as detailed in Section 4.1.1.
The data using MMF with AAV8-hAAT-hFIX16 is described in Section 2.2.5. It may be appropriate to utilize immunosuppressive regimens that may prevent vector-induced hepatitis with MMF at CCI MMF CCI [redacted] There is extensive experience with the proposed immunomodulatory regimens, for example in people with asthma, idiopathic thrombocytopenic purpura, and other medical conditions. For participants with severe hemophilia A, the benefit of long-term expression of a modest level of clotting factor outweighs the risk of a several-week course of immunosuppression. In Cohort 1, CCI [redacted]

4.1.3.4 Experience with Long Term Immunomodulation

There is extensive experience with corticosteroids and immunosuppressive regimens in hemophilia: first, as a manoeuvre 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 hemophilia (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. CCI of steroids has been shown to be efficacious in reducing an immediate immune response with a reduction in corticosteroid related AEs (when compared to daily high dose oral corticosteroids) in several autoimmune diseases (Fanouriakis, 2019; Gordon, 2018; Wei, 2016). The use of CCI [redacted] CCI 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. Participants 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., azathioprine, MMF, and/or tacrolimus 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.). Even though there is clinical experience with the use of these drugs in HIV positive patients, immunomodulatory potential of TCZ in cohort 2 will first be evaluated in 2 participants who are HIV negative.

4.1.4 Screening

Participants who have provided consent will undergo the screening assessments described in the Schedule of Events, up to 16 weeks prior to the infusion of SPK-8011. 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.

4.1.5 Three to Seven Days Prior to Day 0

Participants in Cohorts 1 and 2 will require a laboratory assessment 3 to 7 days prior to Day 0 to verify continued eligibility CCI [redacted] (see Schedule of Events)). 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 CCI [redacted].

4.1.6 Day -2

Participants in Cohort 1 will start CCI [redacted]

4.1.7 Dosing Day (Days 0, 1)

Assessments and procedures to be performed on Dosing Day (Day 0) are described in the Schedule of Events. 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 participant's Infusion Log. Site assistance is permitted for the FVIII infusion. On Day 0, participants in Cohort 2 will then receive a dose of CCI [redacted]

CCI [redacted]. The TCZ dose will not exceed CCI [redacted]. (Please see Actemra® Prescribing Information [Genentech, 2019]). Participants will receive a single IV infusion of SPK-8011 at the vector-administration center. The complete dose of SPK-8011 will be infused via infusion pump over approximately 60 (±2) min. Vital signs will be taken at various time-points from the start of infusion (see Schedule of Events).

Blood samples for immune profiling will be collected pre-administration of TCZ or MMF (CC [redacted]), and pre-administration of SPK-8011, and 30 (±2) min, 2 hours (±10 min), 5 hours (±10 min), and 24 (±1) hours after the completion of the SPK-8011 infusion. PAX Gene will be collected pre-administration of TCZ or MMF (3-7 days prior to Day 0), pre-administration of SPK-8011, 5 hours (±10 min) and 24 (±1) hours after the completion of the SPK-8011 infusion. Additional blood samples, including for FVIII activity levels, will also be collected pre- and post-infusion of SPK-8011.

4.1.8 Follow-up Observation Period

Participants who have received a dose of SPK-8011 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-8011. 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, MMF and TCZ, respectively, may be continued as described in Section 4.1.3.3. In the event that serious infections or malignancies such as lymphoma occur and are assessed as possibly related to either MMF or TCZ, no re-administration of MMF or TCZ shall be allowed to the participant.

4.2 Study Duration, Enrollment and Number of Sites

4.2.1 Duration of Study Participation

The study will consist of the following phases:

- Screening period (up to 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-8011)

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

4.2.2 Total Number of Participants; Sites Projected and Geographic Regions

It is estimated that approximately up to 100 potential participants may be enrolled for a maximum of 50 evaluable (i.e., dosed) participants.

The study is planned to be conducted at approximately 20 study centers (vector-administration centers and/or follow-up centers) located worldwide.

4.3 End of Study Definition

A participant is considered to have completed the study if he has completed all phases of the study including the End of Study (EOS) visit in the Schedule of Events.

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

4.4 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-8011 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-8011 will wait for DMC recommendation. The scheduled date for SPK-8011 infusion may be postponed or cancelled.

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

- Any SPK-8011 related death during the study;
- The development, in any participant, of SPK-8011 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-8011 related Grade IV (> 10 x ULN) elevated transaminases;
- The development, in any participant, of SPK-8011 related Grade I or higher (≥ 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-8011-101 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 time frame according to Section 9.4.2 of the protocol. 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 the following criteria at screening and prior to dosing of SPK-8011 (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 EDs to any recombinant and/or pd 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. 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 remote (greater than 5 years) documentation 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 TCZ in Cohort 2

8. Agree to use reliable barrier contraception after the administration of SPK-8011 until notified by the Investigator or designee.

5.2 Exclusion Criteria

If any of the following criteria exist at screening or prior to dosing of SPK-8011 (Day 0) participants 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 at screening for each of the following tests: HBsAg (hepatitis B surface antigen), anti-HBc (total hepatitis B core antibody), and a 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. Participants treated with anti-viral therapy for chronic hepatitis C must have completed anti-viral therapy at least 6 months prior to screening and have a negative HCV-RNA at the time of screening.

      iii. Participants with a documented or self-reported history of HCV must have a single negative HCV-RNA at time of screening;

2. 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:

3a. Serum albumin measurement. A participant is not eligible if the serum albumin level is below the lower limit of normal of the laboratory; ***and***

3b. A 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. AST-Platelet Ratio Index (APRI) > 1;

If more than 1 diagnostic test result is available, then the FibroScan 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. Participants who are HIV-positive and stable, with an adequate $CD4^+$ count (> 200/mm^3) and undetectable viral load (< 50 gc/mL) measured twice in the 6 months prior to enrollment, and who are on an antiretroviral drug regimen, are eligible to enroll;
5. Have anti-AAV-Spark200 neutralizing antibody titers ≥ 1:1;
6. Have 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 consider to constitute an unacceptable risk;
7. Have been dosed in a previous gene therapy research trial within the last 52 weeks or participated in a clinical study with an investigational drug within the last 12 weeks prior to signing the informed consent;
8. History of diverticulitis, diverticulosis requiring antibiotic treatment, or chronic ulcerative lower gastrointestinal disease that might predispose a patient to perforations;
9. Any concurrent clinically significant major disease (such as liver abnormalities or type I diabetes, uncontrolled hypertension, 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. 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 consideration of the potential clinical relevance of additional screening;
10. Planned surgical procedure in the next 12 months requiring FVIII prophylactic treatment; and
11. Unable or unwilling to comply with the schedule of visits and study assessments described in the clinical protocol.

Participants who do not meet all the enrollment 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 screen failure 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 received from the Sponsor.

5.4 Enrollment of Participants

Each participant will be assigned a unique participant identification (ID) number after the 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. Participant ID numbers will be used to identify the participant for the duration of the study and may not be reassigned to another participant.

5.5 Randomization

Not applicable. This is a non-randomized study.

5.6 Blinding Procedures

Not applicable. This is an open-label study.

6 STUDY PROCEDURES/ASSESSMENTS AND SCHEDULE

6.1 Safety Assessments

6.1.1 Clinical Safety Assessments

The following clinical assessments will be performed to assess the safety profile of SPK-8011:

- Physical examination
- Hemophilia medical history for the 52 weeks preceding screening (including number of FVIII infusions and number of spontaneous and traumatic bleeding episodes).
- Target joint history for the 52 weeks preceding screening. Joint status will be evaluated and assessed during this study using hemophilia joint health score.
- Vital signs
- AEs
- Concomitant therapies and procedures.

6.1.2 Laboratory Safety Assessments

The following laboratory tests will be performed to assess the safety profile of SPK-8011:

- Hematology
- Clinical chemistry
- Liver function tests
- Coagulation
- Neutralizing antibody development against FVIII by Bethesda assay (FVIII inhibitor)
- Neutralizing antibody to AAV-Spark200
- PBMCs to assess cellular immune responses
- Inflammatory profiling of plasma after vector administration
- Vector shedding (PBMC, 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 the screening or pre-vector infusion. The Hemophilia Joint Health Score (HJHS) 2.1 will be performed to evaluate the clinical status of the joints at screening or prior to Day 0, Week 26 and Week 52.

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 HJHS (see Appendix 13.5). 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 (Trakymiene, 2010).

Six joints (left ankle-LA, right ankle-RA, left elbow-LE, right elbow-RE, left knee-LK, right knee) 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 (Changes in Level of Activity and Hemophilia Activities List)

Changes in Level of Activity will be assessed at selected visits. The participant will review the following 3 statements to confirm which more accurately describes any changes in his amount and intensity of physical activities in the past month.

a) I have been doing more amount and more intensive physical activities.

b) I have been doing fewer amount and less intensive physical activities.

c) I have been doing about the same amount and intensity of physical activities.

In addition to the Changes in Level of Activity, participants will complete the Hemophilia Activities List questionnaire (see Appendix 13.2) (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 Appendix 13.3): 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 Appendix 13.4): 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 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 immunology, further coagulation assays, future research, or for clarification of any clinical or laboratory AEs. If a participant provides consent, samples may be used for genetic analyses.

6.3 Clinical Procedures

The clinical procedures that will be conducted during this study related to the evaluation of population demographics and safety are provided in Table 1.

Table 1 Clinical Procedures: Safety and Efficacy 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 FVIII therapy in the past 52 weeks
Pharmacokinetic Profile	Current FVIII product. • Recovery (Cmax following a known IU/kg dose of FVIII) • Half-life calculations
Joint Assessments	Target joint assessment: • Location of target joint(s) • Location of joint bleeding • Frequency of bleed per joint • Target joint history in the past 26 weeks HJHS

Assessment	Description
Physical Exam	Full physical examination • Hemophilic arthropathy assessment Height and body weight
Vital Signs	Blood pressure (systolic and diastolic) Respiratory rate Pulse rate Temporal or oral temperature (°C)
ECG	ECG
Quality of Life Questionnaires	Haem-A-QoL EQ-5D-5L
Activity Assessments and Health Economics	Hemophilia Activities List Health Economic Assessment
Review of Adverse Events and Concomitant Therapies/Procedures	Review of adverse events and concomitant therapies/procedures throughout the study
Hematology (CL, LL)	WBC count with Differential ○ Neutrophils ○ Lymphocytes ○ Monocytes ○ Eosinophils ○ Basophils ○ ANC (screening Only) 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 (LL) • Liver ultrasound • α-fetoprotein (CL)
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 only at screening), VWF antigen (CL), VWF activity (CL) • Spare plasma (CL)

Assessment	Description
Neutralizing Antibody (CL)	rAAV-CCI neutralizing antibody
Immunology (CL)	• Immun PBMC for ELISpot • Immun PBMC for Inflamm

Immune Profiling (CL)	• Inflammatory profiling of plasma after vector administration
	  ○ Cytokine/Chemokine analysis
	  ○ Tryptophan metabolites
	  ○ Complement activation

PAX Gene (CL)	• PAX Gene (RNA)
Vector Shedding (CL)	• PBMC • Saliva • Serum • Urine • Semen
Hepatitis (CL)	• Hepatitis B surface antigen • HCV RNA Ampliprep Taqman 2.0 • Hepatitis B core antibody total • HBV DNA Ampliprep Taqman 2.0 • HCV antibody
HIV Serology (CL)	HIV-1/HIV-2 antibody and antigen screen
Viral Load and $CD4^+$ (CL)	HIV-1/HIV-2 qualitative RNA, $CD4^+$ count
TPMT (LL)	TPMT activity or genotype
Lipid Profile (CL, LL)	Lipid profile (total cholesterol, LDL, VLDL, HDL, triglycerides)
TB Testing (LL)	Tuberculosis Interferon-gamma Release Assay
SARS-CoV2 Testing (LL)	Anti-SARS-CoV2 serology NAAT for infection with SARS-CoV2
Fibrinogen (LL)	Fibrinogen antigen, D-dimer, thrombin time

ALP = alkaline phosphatase, ALT = alanine aminotransferase, ANC = absolute neutrophil count, aPTT = activated partial thromboplastin time, AST = aspartate aminotransferase, BUN = blood urea nitrogen, CL = central lab (as per Laboratory Manual), $CD4^+$ = cluster of differentiation 4, Cmax = maximum drug concentration, CRP = C-reactive protein, ECG = electrocardiogram, ELISpot = enzyme-linked immunospot assay, EQ-5D-5L = EuroQol- 5 Dimension 5 Level, FVIII = coagulation factor VIII, GGT = gamma-glutamyl transferase, Haem-A-QoL = Haemophilia A Quality of Life, HBV = hepatitis B virus, HCV = hepatitis C virus, HDL = high-density lipoprotein, HIV = human immunodeficiency virus, HJHS = Hemophilia Joint Health Score, HLA = human leukocyte antigen, LDH = lactate dehydrogenase, LDL = low-density lipoprotein, LL = local lab, NAAT = nucleic acid amplification testing, PBMC = peripheral blood mononuclear cells, rAAV = recombinant adeno-associated viral vector, RBC = red blood cell, SARS-CoV2 = severe acute respiratory syndrome coronavirus 2, TB = tuberculosis, TPMT = thiopurine methyltransferase, VLDL = very low-density lipoprotein, VWF = von Willerbrand 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. At the time of consent, participants will be enrolled into the study. 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-8011-101 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 planned day of administration of SPK-8011.

Anti-SARS-CoV2 serology testing will be conducted at screening 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, which may occur over a period up to 16 weeks:

- Obtain written informed consent
- Review inclusion/exclusion criteria
- Review prior and concomitant therapies/procedures
- Record AEs
- Record information on demographic and baseline characteristics
- Record medical history and hemophilia history, including FVIII treatment history and bleeding history
- Record target Joints, HJHS, and joint health assessment – *May be done between screening and prior to Day 0*
- Measure vital signs (blood pressure, pulse, respiratory rate, and oral/temporal temperature)
- Perform physical examination, including height and weight
- Haem-A-QoL, EQ-5D-5L, Hemophilia Activities List, Health Economic Assessment – *May be done between screening and prior to Day 0*
- Obtain samples for central laboratory evaluation, unless otherwise noted:
    - Hematology
    - Clinical chemistry
    - α-fetoprotein
    - Vector shedding (PBMC, serum, saliva, urine, semen) – *May be done between screening and Day 0*
    - Spare plasma
    - AAV neutralizing antibody
    - HBsAg, anti-HBc, HBV-DNA
    - HCV-RNA load assay

  - HIV-1/HIV-2 antigen/antibody
  - Urinalysis
  - 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, FibroTest/FibroSURE, or AST-to-Platelet Ratio Index *(for participants without known pre-existing diagnosis of portal hypertension, splenomegaly, or hepatic encephalopathy) (local lab or central lab)*
  - Fibrinogen *(local lab)*
    - Antigen
    - D-dimer
    - Thrombin time
  - Thiopurine methyltransferase (TPMT) *(local lab)*
  - TB Interferon-gamma Release Assay *(local lab)*
  - Anti-SARS-CoV2 serology *(local lab)*
- If indicated:
  - HIV-1/HIV-2 viral load and $CD4^+$ T cell count *(for participants who are HIV-positive), (central lab),*
  - 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".)*
  - Electrocardiogram (ECG) *(for participant > 50 years of age, or if clinically indicated)*
  - Lipid panel *(for participants with a history of dyslipidemia or hypercholesterolemia, or if clinically indicated) (central lab)*
  - 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 (Cmax 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 at Screening or Day 0 – *May be done between screening and Day 0*

*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 assessment should occur within 3 to 7 days prior to Day 0 for Cohort 2. Results must be consistent with inclusion/exclusion criteria.

- Liver function test, CRP *(local lab)*
- Hematology *(local lab)*
- Immunology *(central lab)*
- Immune profiling *(central lab)*
- PAX gene (RNA) *(central lab)*
- SARS-CoV2 NAAT testing *(local lab)*

At the discretion of the investigator, NAAT for infection with SARS-CoV2 (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/recurrent muscle pain 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-CoV2 RNA testing in symptomatic individuals with URTI or ILI suspected of having COVID-19.

The participants in Cohort 1 should be contacted prior to CCI to verify no changes in the general health status and that laboratory assessments collected prior to MMF dosing are consistent with inclusion/exclusion criteria, and to confirm the prescribed MMF regimen.

6.6 Day -2

On Day -2, participants in Cohort 1 will begin taking oral MMF CCI .

6.7 Dosing Day Assessments (Days 0, 1)

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 participant's Infusion Log. Supervision from the site staff is permitted.

Cohort 2 only: On Day 0, participants in Cohort 2 will receive a dose of CCI

All participants will be infused with a single IV infusion of SPK-8011 at the vector-administration center. The complete dose of SPK-8011 will be infused via infusion pump over approximately 60 (±2) min.

6.7.1 FVIII Dosing

- Verify the administration (self-infused or assisted by site staff) of a single bolus of 50 IU/kg of FVIII product the morning of CCI or prior to TCZ in Cohort 2.

6.7.2 Tocilizumab (TCZ) Dosing

- Verify no changes in the general health status, and that laboratory assessments collected up to 1 week prior to TCZ dosing are consistent with inclusion/exclusion criteria.

6.7.3 Vector Dosing

*Assessments **before** SPK-8011 Vector infusion:*

- Review inclusion/exclusion criteria
- Measure vital signs (blood pressure, pulse, respiratory rate, and oral/temporal temperature)
- Perform physical examination, including weight
- Obtain samples for central laboratory evaluation, unless otherwise noted:
  - Coagulation:
    - aPTT and FVIII Activity *(local and central labs)*
    - FVIII antigen
  - Immunology
  - Immune profiling
  - PAX gene (RNA)
- Record AEs and concomitant therapies/procedures

*Assessments **during** Vector infusion:*

- Administer a single IV infusion of SPK-8011 via infusion pump over approximately 60 (±2) min
- Record AEs and concomitant therapies/procedures

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

- Vital signs immediately following the infusion (±2 min), and 2 hours (±10 min) after the end of vector infusion
- Obtain blood samples for central laboratory evaluation from the completion of the vector infusion:
  - Immune profiling at 30 (±2) min, 2 hours (±10 min), and 5 hours (±10 min)
  - PAX gene (RNA) at 5 hours (±10 min)

- Record AEs and concomitant therapies/procedures

6.7.4 Day 1

Measure vital signs 24 (±1) hours post-SPK-8011 dose (blood pressure, pulse, respiratory rate, and oral/temporal temperature)

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

- Immunology
- Immune profiling (24 (±1) hours post vector infusion)
- PAX gene (24 (±1) hours post vector infusion)
- Hematology (*local and central labs*)
- Coagulation (aPTT and FVIII activity (*local and central labs*), FVIII antigen)
- Liver function tests and CRP (*local and central labs*)
- Spare plasma
- Record AEs and concomitant therapies/procedures

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 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. *Guidance for Industry, Investigators, and Institutional Review Boards on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic* (U.S. Food and Drug Administration, 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 Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16 (±2 days)

A minimum of twice weekly visits are required from Weeks 1-11 to monitor for a potential rise in hepatic transaminases and/or loss of FVIII transgene expression. Visits at Weeks 1-11B may be performed by an in-home service provider. Visits will occur every 2 weeks from Weeks 12-16.

If an apparent immune response/liver inflammation is observed and triggers the initiation of reactive corticosteroids, please collect additional samples for immune profiling and PAX gene prior to corticosteroid administration, if possible (see Schedule of Events and Section 4.1.3.1).

If immunomodulation is initiated, weekly visits should continue to monitor transaminases, FVIII (local and central lab), and ELISpot. Weekly hematology and monthly lipid profile results should also be monitored locally for Cohorts 1 and 2 until MMF or TCZ dosing has stopped; 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 *(Weeks 1A, 7A, 12)*
- Measure vital signs *(Weeks 1A, 7A, 12, 14, 16)*
- Hemophilia Activities List, Health Economic Assessment *(Weeks 7A, 12, 14)*
- Review FVIII Infusion Log *(Weeks 7A, 12, 14)*
- Obtain samples for central laboratory evaluation at all visits, unless otherwise noted:
    - Coagulation
        - aPTT, FVIII activity *(local and central labs)*
        - FVIII antigen (*central lab*)
    - Liver function tests and CRP *(local and central labs)*
    - Hematology *(Weeks 1A, 7A, 12, 14, 16) (local and central labs)*
    - Clinical chemistry *(Weeks 1A, 7A, 12)*
    - HBsAg, anti-HBc (*Week 12*)
    - AAV neutralizing antibody *(Weeks 1A, 2A, 4A, 7A, 12, 14)*
    - Immunology (*Weeks 1-11A, 12, 14, 16)*
    - Immune profiling *(Weeks 1-4B, 8B, 16)*
    - PAX gene (RNA) *(Weeks 2B, 4B, 8B)*
    - Spare plasma *(Week 12)*
    - Vector shedding samples (PBMC, 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 *(Weeks 2A, 4A) (local lab)*
    - Fibrinogen antigen
    - D-dimer
    - Thrombin time
- Record AEs and concomitant therapies/procedures

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

Visits will occur 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 monitor transaminases, FVIII, and ELISpot. Hematology and lipid profile results should also be monitored regularly for Cohorts 1 and 2 until MMF or TCZ dosing has stopped. This can 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 – if indicated*)
- Record target joints, joint health assessment, HJHS *(Weeks 26, 52/EOS)*

- Hemophilia Activities List, Health Economic Assessment *(Weeks 26, 40, 52/EOS)*
- Haem-A-QoL, EQ-5D-5L *(Weeks 26, 52/EOS)*
- Review FVIII Infusion Log *(Weeks 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, VWF activity, VWF antigen (*Week 52/EOS only)*
    - Liver function tests and CRP *(local and central labs)*
    - Immunology
    - Lipid panel (*Week 52/EOS Only*)
    - Vector shedding samples (PBMC, serum, saliva, urine, semen)
        - NOTE: Vector shedding samples are collected only until 3 consecutive samples are negative
    - Hematology *(Weeks 18, 26, 52/EOS)*
    - Clinical chemistry *(Weeks 18, 26, 52/EOS)*
    - AAV neutralizing antibody *(Weeks 26, 40, 52/EOS)*
    - Spare plasma *(Weeks 26, 40, 52/EOS)*
    - Urinalysis *(Weeks 26, 52/EOS)*
    - Anti SARS-CoV2 serology *(Week 18)(local lab)*
    - α-fetoprotein (*Week 52/EOS*)
- Record AEs and concomitant therapies/procedures

Week 52 procedures are to be performed only at the EOS Visit. If LTFU study is not open at the clinical site at the time of a participant's planned Week 52 visit, the participant may remain in Study SPK-8011-101. 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-8011
Formulation (including dosage form and strength):	CCI [redacted]
Route of Administration:	IV infusion
Packaging and Labeling:	CCI [redacted] Study drug will be labeled as required per country requirements.

CCI [redacted]

Refer to the most recent version of the Investigator and Pharmacy Brochures for further details on the study drug.

7.2 Study Doses

The proposed SPK-8011 doses for this study:

- Starting dose ($5x10^{11}$ vg/kg)
- Middle dose ($1x10^{12}$ vg/kg)
- High dose ($2x10^{12}$ vg/kg)
- Intermediate Dose ($1.5 x10^{12}$ vg/kg)

After escalation through the first 3 planned dose levels, an intermediate dose level (e.g., $1.5x10^{12}$ vg/kg of SPK-8011), was agreed with the DMC Any decision to add an intermediate 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-8011. 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

Refer to Figure 1 for a description of the sequence of enrollment. In Cohort 1, oral MMF will be self-administered by the participant starting on CCI per the product insert. On Day 0, the participant will prophylactically infuse 50 IU/kg of the participant's usual FVIII protein product per the product insert. In Cohort 2, this infusion of FVIII protein will occur first CCI per the product insert. For both cohorts, after these steps, the participant will be infused with SPK-8011 intravenously over approximately 60 (±2) min via infusion pump.

7.4 Treatment Compliance

Compliance with the infusion of FVIII protein product and SPK-8011 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 are monitored and recorded by the site staff.

Participants in Cohort 1 will initiate MMF approximately 48 hours prior to CCI The time of administration will be monitored and recorded by the site staff. Participants in Cohort 2 will be infused with CCI via an infusion pump and will be supervised by the study staff. The lot number, total volume infused, and infusion time are monitored and recorded by the site staff.

After the infusion of FVIII protein product and at least 4 hours (and up to 24 hours) after TCZ (Cohort 2 only) participants will be infused with SPK-8011 CCI. SPK-8011 will be infused via infusion pump and will be supervised by the investigational staff. The lot number, total volume infused, and infusion time are monitored and recorded by the site staff. Therefore, full compliance with SPK-8011 infusion is anticipated.

7.5 Study Drug Storage

SPK-8011 must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions CCI with access limited to the investigator and authorized site staff.

7.6 Study Drug Preparation, Handling and Disposal

7.6.1 Study Drug Preparation

Prior to preparing the study drug, the responsible pharmacy staff should first carefully review the instructions provided in the Pharmacy Brochure. The investigational pharmacy personnel preparing the study drug for infusion will use universal precautions and appropriate personal protective equipment. The dilution will be performed using aseptic techniques in a biosafety cabinet. Just prior to use, the frozen product will be thawed at room temperature and diluted according to the verified dose calculation worksheet. Once the vials are diluted, SPK-8011 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 for all study drug received and any discrepancies are reported and resolved before use of the study drug. Immediately after receipt, the study drug should be stored as described in Section 7.5.

Only participants enrolled in the study may receive study drug and only a trained and authorized site staff may supply or administer the IMP. Study drug must only be thawed after confirmation that the participant is eligible, and the appropriate washout period was met, if applicable. Vials are for single use only.

The site should store all used and unused vials of SPK-8011, 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 disposition records).

All vials, both used and unused, must be retained for monitoring and study drug accountability. At study completion, reconciliation must be made between the amount of study drug supplied, dispensed, and subsequently destroyed or returned to the Sponsor, or its designee.

Further guidance and information for the final disposition of used/unused study drug are provided in the Pharmacy Binder.

7.7 Labeling

The product label includes: Investigational product name; manufacturer; specific lot number; date of manufacture; vial number; storage instructions; and investigational product warning. The Pharmacy Brochure will contain copies of approved labels.

7.8 Study Compliance

Following the administration of SPK-8011, participants will be followed according to the protocol Schedule of Events. 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 receives 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. 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 hepatotoxicity 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.
- Non-steroidal anti-inflammatory drugs, **except for** 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 the health of your liver 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 is prohibited unless clinically indicated and in discussion with the investigator and sponsor
- Any other investigational therapies prior to screen as defined in the exclusion criteria or used during the study

Any concomitant medication that has known hepatotoxicity should be discontinued in the first 12 weeks following vector infusion. 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 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 his 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.

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-8011), 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 study procedures to be done 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 (see the Schedule of Events).

9 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The definitions of an AE or SAE can be found below.

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 that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study (see Section 8).

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 procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

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:

- **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.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

- **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 situations**:

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

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

- The prescheduled, or elective procedure, or routinely scheduled treatment was scheduled (or 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 as adverse events of special interest (AESIs) to enable an adequate risk-benefit evaluation of SPK-8011 versus standard therapy during the study and additional data may be requested for these events. The AESIs are:

- AEs associated with FVIII inhibitor formation
- The development, in any participant, of SPK-8011 related Grade IV (> 10 x ULN) elevated transaminases

- The development, in any participant, of SPK-8011 related Grade I or higher (≥ 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-8011 or one of the immune modulating agents
- Thrombotic and/or embolic events (TEE)

9.2 Recording and Follow-Up 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 CRF.
- 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 CRF 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.

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

9.3 Safety Classifications

9.3.1 Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 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 outcomes as described in the definition of an SAE, NOT when it is rated as 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 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 make an assessment of causality for every event before the initial transmission of the SAE data to Spark or designee.

The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

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 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 CRF.

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 report form are as follows:

- All AEs must be recorded in the CRF in a timely manner.
- All SAEs must be reported in the SAE report 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 the phone conversation with medical monitor, this SAE information must be documented and send it via email to Spark PV at sparkpv@sparktx.com immediately. A copy of the SAE report form must be submitted followed by the telephone conversation to SAE reporting email address.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE report form pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in the SAE report 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

All AEs/SAEs will be collected 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 the start of study intervention but after obtaining informed consent will be recorded on the Medical History section of the eCRF not the AE section.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Section 9.4.2. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE 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 the study intervention or study participation, the investigator must promptly notify the sponsor.

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

9.5.1 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.

9.5.2 Pregnancy

Participants participating 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 collecting 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-8011 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-8011 gene therapy product is a one-time administration with no participant access to the study drug. Furthermore, SPK-8011 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 AE(s) is associated with ("results from") the overdose, the AE(s) and overdose will be reported as and SAE, 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 AE as "accidental overdose without adverse effect". All overdoses with and without an AE should be recorded in the eCRF and only overdose with associated AEs should be reported in an SAE report 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 liver function test (LFT) abnormalities. LFT abnormalities 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 CRF.

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-8011.

10.2 Sample Size Determination

The sample size is based on the need to establish the initial safety and efficacy profile of SPK-8011. Up to 50 eligible participants will be dosed. If more than 50 eligible participants are to be dosed, regulator(s) will be informed along with the DMC.

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 Analysis Population

The Full Analysis Set (FAS) is defined as all participants who receive the infusion of SPK-8011. The analyses of safety and efficacy will be performed in this population, including the evaluation of vector-derived FVIII:C activity levels 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-8011 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 each 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, vector shedding analysis, and physical examination findings will be summarized by descriptive statistics. All AEs will be tabulated by occurrence, grouped by body system, and summarized by dose group. The rate of occurrence for each AE will be summarized by body system, severity, and relation to the administration of SPK-8011. 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., LFT abnormalities) 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.).

10.5.2 Efficacy Analysis

Summary statistics will be created by SPK-8011 dose group for the following parameters:

- The number of bleeding events expressed as the ABR per participant (spontaneous and traumatic combined) following SPK-8011 administration and beginning 28 days following SPK-8011 administration.
- Absolute change in ABR for all bleeding events and bleeding events observed beginning 28 days following SPK-8011 administration from the hemophilia history-based ABR for the 52-weeks prior to SPK-8011 administration.
- Proportion of participants with a post-SPK-8011 administration ABR of 0 or 1 (separately for all bleeding events and bleeding events reported beginning at least 28 days following SPK-8011 administration).
- Number of recorded FVIII infusions per participant (referred to in the protocol as annualized FVIII usage) following SPK-8011 administration and beginning 28 days following SPK-8011 administration.
- Absolute change in the number of recorded FVIII infusions following SPK-8011 administration (all infusions and infusions reported beginning 28 days following SPK-8011 administration) from the prior 52-week hemophilia history.

10.5.3 Pharmacokinetic 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-8011 administration will be summarized descriptively by SPK-8011 dose group. For each participant, the difference between the lab visit date and the SPK-8011 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 will 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-8011 administration will be summarized descriptively by SPK-8011 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 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-8011 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, TCZ is expected not to influence levels of the 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 FVIII: activity resulting from the SPK-8011 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 TCZ 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-8011 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, hemophilia joint health score, changes in level of activity, hemophilia activity list, Haem-A-QoL and EQ-5D-5L questionnaires will be summarized descriptively and provided in participant listings. The questionnaire will be scored according to the recommendations of the questionnaire authors. Each domain score and change from baseline in domain score will be summarized by visit.

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) will be summarized descriptively by SPK-8011 dose group.

10.7 Interim Analyses

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

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, applicable ICH GCP guidelines, and 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 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, International Conference on Harmonisation (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 representative will explain the nature of the study to the participant or his/her 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, International Conference on Harmonisation (ICH) guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, 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(s) 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 ICF for pregnancy outcome information.

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., Health Insurance Portability and Accountability Act (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 not be transferred.

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 law(s). The level of disclosure must also be explained to the participant.

11.1.5 Committees 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 other data (as needed) on a regular basis during 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 electronic CRF 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 CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- 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 CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 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 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 sites will be closed upon study completion. 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 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 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 the right of the Investigator, while free to utilize data derived from the study for scientific purposes or publish the results in recognized scientific journals, is 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, except that Investigator(s) shall delete any confidential information from such proposed publication.

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13 APPENDIX

13.1 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)

13.2 Hemophilia Activities List and Change in Level of Activity

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

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

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

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

Change in Level of Activity –

Questionnaire

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

In the previous month, which of the following statements is most accurate:

O I have been doing more amount and more intensive physical activities.

O I have been doing fewer amount and less intensive physical activities.

O I have been doing about the same amount and intensity of physical activities.

Thank you for completing both HAL and Change in Level of Activity questionnaires.

Thank you very much for your cooperation.

13.3 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!

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 -

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	☐	☐	☐	☐	☐

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 –

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	☐	☐	☐	☐	☐	☐

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 -

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	☐	☐	☐	☐	☐

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 -

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	☐	☐	☐	☐	☐

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 -

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	☐	☐	☐	☐	☐	☐

HAEM-A- QOL - USA/English - Final version - 12 Oct 15 -

 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!

13.4 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 ☐

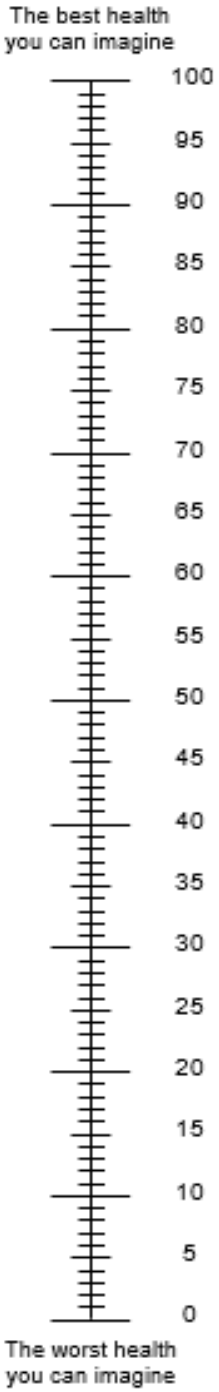
ANXIETY / DEPRESSION

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 ☐

2

- 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 = ☐



3

13.5 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: Measured in: 1) Supine ☐ 2) Sitting ☐	Flex: Measured in: 1) Supine ☐ 2) Sitting ☐	Flex: Measured in: 1) Supine ☐ 2) Sitting ☐	Flex: Measured in: 1) Supine ☐ 2) Sitting ☐	PlantarFlex: Measured in: 1) Supine ☐ 2) Sitting ☐	PlantarFlex: 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) **Hyperextension**: record as "plus" (+) ____ degrees **Loss of extension** record as "minus" (-) ____ degrees	Ext: Measured in: 1) Supine ☐ 2) Sitting ☐	Ext: Measured in: 1) Supine ☐ 2) Sitting ☐	Ext: Measured in: 1) Supine ☐ 2) Sitting ☐	Ext: Measured in: 1) Supine ☐ 2) Sitting ☐	DorsiFlex: Measured in: 1) Supine ☐ 2) Sitting ☐	DorsiFlex: 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°

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

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13.6 Abbreviations

AASLD	American Association for the Study of Liver Diseases
AAV	Adeno-associated virus
AAV2	Adeno-associated virus, serotype 2
AAV5	Adeno-associated virus, serotype 5
AAV8	Adeno-associated virus, serotype 8
AAVrh10	Adeno-associated virus, rhesus serotype 10
AAV-Spark100	Adeno-associated virus Spark100 vector
AAV-Spark200	Adeno-associated virus Spark200 vector
ABO	Blood type
ABR	Annualized bleeding rate
AE	Adverse event
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
aPTT	Activated partial thromboplastin time
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase
BDD	B-domain-deleted
BDD-hFVIII	B-domain-deleted human factor VIII
BID	Twice a day
BMI	Body mass index
BMN-270	Adeno-associated viral vector, serotype 5, with B-domain-delated human factor VIII gene (aka AAV5-BDD-hFVIII)
bp	Base pair
BP	Blood pressure
BQL	Below quantifiable limits
BU	Bethesda units
BUN	Blood urea nitrogen
˚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
Cl	Chlorine
CNS	Central nervous system
COVID-19	Coronavirus disease caused by SARS-CoV2
CRF	Case report form
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
ECG	Electrocardiogram
CRF/ eCRF	Case report form
ED	Exposure day
EHL	Extended half-life
ELISpot	Enzyme-linked immunospot assay
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
FK506	Tacrolimus
FVIII	Coagulation factor VIII
FVIII:C	Factor VIII in circulation
FVIII-SQ	The "SQ" form of B-domain deleted human coagulation factor VIII as described by Lind (Lind, 1995)
FXa	Activated factor X
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
Haem-A-QoL	
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High-density lipoprotein
hFIX	Human coagulation factor IX
hFIX39-Padua	Human coagulation factor IX Padua variant
hFVIII	Human coagulation factor VIII
HIV	Human immunodeficiency virus
HJHS	Hemophilia Joint Health Score
HLA	Human leukocyte antigen
HRQoL	Health-related quality-of-life
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

IgG4	Immunoglobulin G subclass 4
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
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
MMF	Mycophenolate mofetil
mRNA	Messenger ribonucleic acid
nAb	Neutralizing antibody
NAT	Nucleic acid test
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
pd	Plasma derived
PHI	Protected health information
PI	Principal Investigator
PK	Pharmacokinetics
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	Severe acute respiratory syndrome coronavirus
SD	Standard deviation
SPK-8011	Study drug in this protocol Adeno-associated viral Spark200 vector with B-domain-delated human factor VIII gene (aka. AAV-Spark200-BDD-hFVIII)
SPK-9001	Adeno-associated viral vector comprised of the AAV-Spark100 capsid, and

	a codon-optimized FIX mini-gene encoding human FIX-Padua (aka. AAV-Spark100-hFIX39- Padua)
TCZ	Tocilizumab
TEAE	Treatment emergent adverse events
TEE	Thrombotic and/or embolic events
TTR	Transthyretin
TPMT	Thiopurine Methyltransferase
TTRm	Modified transthyretin
ULN	Upper limit of normal
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

13.7 Protocol Amendment History

The Protocol Amendment Summary of Changes Table for all amendments are located below.

DOCUMENT HISTORY	
Document	**Date**
Amendment 7	09-Feb-2021
Amendment 6	29-Sep-2020
Amendment 5	27-Jan-2020
Amendment 4	08-Nov-2018
Amendment 3	09-Aug-2018
Amendment 2	24-Aug-2017
Amendment 1	21-Nov-2016
Original Protocol	17-Oct-2016

SUMMARY OF CHANGES FROM PREVIOUS VERSION:

SECTION OF AMENDMENT 7:	SUMMARY OF REVISIONS MADE:	RATIONALE:
Throughout the document	Use new protocol template	To align with updated template per Spark SOP
1.1 Protocol Synopsis	Protocol Synopsis has been updated.	Updates reflect changes made throughout Amendment 7.
1.2 Schema	Updated Figure 1 (Study Schematic) to revise the immunomodulatory regimens for Cohorts 1 and 2.	The study schematic was updated to replace reactive corticosteroid regimen with prophylactic MMF
1.3 Schedule of Events	Updated Schedule of Events • Added ○ Review of Inclusion/Exclusion criteria prior to vector dosing ○ Laboratory collections at screening and 3 to 7 days prior to vector infusion ○ Prophylactic MMF dosing and remove reactive corticosteroid regimen ○ CCI infusion of MMF for Cohort 1. ○ Screening and follow-up testing for immunomodulation ○ Revised assessments/procedures for Day 0 pre-SPK-8011 dosing	Additional screening and follow up is required as well as weekly follow up while on immunomodulation.

	○ Immune profiling to Pre-SPK-8011 Dosing and Weeks 1B, 2-6, 8-11, and 16 ○ Immunology to Day 1 ○ PAX Gene (RNA) to Pre-SPK-8011 Dosing and Weeks 2 to 6 • Removed ○ ABO group from screening ○ Hematology, chemistry, LFT, CRP, immunology, and AAV neutralizing antibody from Pre-SPK-8011 Dosing	
2.2.3	Text related to the immunomodulatory regimens was added.	To provide rationale for the administration of MMF and the use of CCI IV steroids.
2.2.5	Added new section (Summary of Non-clinical and Clinical Experience with Mycophenolate Mofetil)	The preclinical and clinical study results provide evidence to support the design of Cohort 1.
2.3.1.2	Added inhibitor data from ongoing Study SPK-8016-101	To updated inhibitor information based on the available data
2.3.1.3	Removed the appearance of ELISpot signal in peripheral blood as a possible or probable indicator of a cellular immune response for participants with transaminase elevations. Note, text referring to use of this as an indicator of immune response has been removed throughout protocol.	To provide guidance to the investigators based on current data
2.3.1.6	Adverse events occurring in this study and Study SPK-8016-101 were updated.	To update adverse event information based on the available data.
2.3.1.7	Updated to include MMF and to change the timing for review and completion of vaccinations prior to the planned day of SPK-8011 administration, with provisions in timing for participants receiving immunosuppressive medication.	To provide current information based on available data.
2.3.1.7.2	Updated information related to the immunomodulatory regimen and FVIII level for 2 participants administered tacrolimus in this study.	To provide current information based on available data.
2.3.1.7.4	Added new section (Mycophenolate Mofetil) detailing risks associated with this agent.	To provide the risks associated with the addition

		of the immunomodulating agent MMF
2.3.1.7.5	Added MMF and clarified references	To include the immunomodulation agent MMF
4.1.1	Updated to detail immunomodulatory regimens for Cohorts 1 and 2 and add time frame for administration of TCZ for the first 2 participants treated with this agent.	To provide additional guidance based on the addition of the MMF cohort
4.1.3	Updated the details regarding corticosteroid use	To include clinical data based on corticosteroid use as well as provide rationale for removing IV corticosteroids as a cohort
4.1.3.2	Added MMF to section on other immunomodulatory considerations	To include the immunomodulation agent MMF
4.1.3.3	Added details regarding the prophylactic use of TCZ and MMF	To provide details regarding the administration of MMF for participants in Cohort 1
4.1.5	Updated to include MMF immunomodulatory regimen	To provide additional guidance based on the addition of the MMF cohort
4.1.6	Added Day -2	To provide guidance based on the addition of the MMF cohort
4.1.7	Revised/clarified timepoints for sample collection for immune profiling, PAX gene and FVIII activity	To align with Schedule of Events
4.1.8	Updated to include MMF immunomodulatory regimen	To include the immunomodulation agent MMF
4.2.2	Increased number of study centers included in this study	To increase projected number of study centers
6.3	Revised Table 1 to: • add PK assessments • add hemophilic arthropathy assessment • add ECG • add ANC at screening • add liver fibrosis diagnostic tests • add spare plasma collection • clarify HIV assessments	To align with Schedule of Events
6.4.1	Revised screening assessments/procedures to align with Schedule of Events	To align with Schedule of Events
6.5	Added instructions for 3 to 7 days prior to Day 0	To provide additional instructions for MMF cohort

6.6	Added Day -2	To provide guidance based on the addition of the MMF cohort
6.7	Revised Day 0 and Day 1 assessments/procedures to align with Schedule of Events	To align with Schedule of Events
6.8	Revised follow-up observation period (Weeks 1-52) assessments/procedures to align with Schedule of Events	To align with Schedule of Events
7.3	Added/clarified CCI [redacted] instructions on administration of CCI [redacted], FVIII protein and SPK-8011.	To add/clarify dosing administration details
7.4	Added/clarified instructions for treatment compliance with MMF and TCZ	To include the immunomodulation agent MMF
7.9.1 and 7.9.3	Added statement regarding permitted and prohibited therapies	To clarify the types of medications that are permitted/prohibited
10	Reformatted, restructured, and updated statistical considerations	To align with new template format and changes to the protocol
11.1.9	Revised study/site closure instructions	To clarify reasons for study/site closure
13.2	Updated Hemophilia Activities List questionnaire to a more current version	To provide current version of questionnaire

SECTION OF AMENDMENT 6:	SUMMARY OF REVISIONS MADE:	RATIONALE:
1.1 Protocol Synopsis	Protocol Synopsis has been updated.	Updates reflect changes made throughout Amendment 6.
1.2 Schema Figure 1	Updated study schematic figure 1.	The study schematic was updated to include the cohorts in the $1.5x10^{12}$ dose.
1.3 SOE	Updated Schedule of Events	Additional screening and follow up is required for participants in cohort 2 of the $1.5x10^{12}$ dose. This includes screening labs, three to seven days prior to vector, 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 Tocilizumab	The preclinical study results provide evidence to support the design of cohort 2 in the $1.5x10^{12}$ dose group.
2.3.1	Added risks associated with tocilizumab	The risks associated with TCZ were provided to support the design of cohort 2 in the $1.5x10^{12}$ dose group.
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 $1.5x10^{12}$ dose into two cohorts are included. A description of the expansion of cohorts up to 10 participants is described if additional data is needed.
4.1.1	Refined dose cohort expansion.	Details for the expansion and enrollment sequence of the $1.5x10^{12}$ dose 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 50 participants.	The expansion of the $1.5x10^{12}$ dose into three 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 TCZ.
2.2.4 & 2.2.5	Added preclinical experience with tocilizumab	The preclinical study results provide evidence to support the design of cohort 2 in the $1.5x10^{12}$ dose group.
2.3.1	Added risks associated with tocilizumab	The risks associated with TCZ were provided to support the design of cohort 2 in the $1.5x10^{12}$ dose group.
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 of the $1.5x10^{12}$ dose due to the planned use of TCZ. The testing CCI [redacted] 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.
9.1.1	Updated AE language	AE language was updated to include standard toxicology reporting. Clarification of the timeframe to collect AEs was provided.
Throughout	Updated text and references as it relates 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 5 (version 6.0)	1. Updated study schematic figure 1. 2. Removed language requiring prophylactic steroid administration on Week 4. 3. Updated corticosteroid regimen language to include use of CCI corticosteroids in response to an immune response trigger. 4. 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 5. Inserted Thiopurine methyltransferase (TPMT) as a screening requirement. 6. Added risks associated with azathioprine and tacrolimus in section 1.2.3 7. Updated text and references as it relates to immunomodulatory clinical experience in Hemophilia Gene Therapy trials.
Amendment 4 (Version 5.0)	1. Introduced the use of SPK-8011 drug substance produced using a new manufacturing process (see Section 9.1 for additional information). 2. Revised Inclusion criterion #6 to provide further clarity guidance for investigators. 3. Revised Figure 1 to clarify the dose cohort graphic. 4. Removed instruction on signs and symptoms of thrombotic and/or embolic events from the Schedule of Events. 5. Added emicizumab as a prohibited therapy 6. Updated corticosteroid regimen language to allow investigator's discretion in determining if prophylactic corticosteroids should be administered to participants 7. Clarified washout language to include washout period for participants taking non-factor replacement products. 8. Inserted the Change in Level of Activities Questionnaire to Appendix 2. 9. Clarified other potentially confusing text and incorporated administrative changes throughout the protocol.

Document	Description of Main Changes
Amendment 3 (Version 4.0)	1. Introduced a prophylactic corticosteroid regimen (see Section 3.2.4). 2. Deleted bullet regarding steroid use in Section 7.1.2 (Permitted Therapies) as corticosteroids are now required, not permitted. 3. Moved paragraph from Section 3.2.5 regarding long term use of corticosteroids to Section 1.2.3 under Possible Side Effects from Corticosteroids. 4. Updated Schedule of Events to include prophylactic steroid regimen. 5. Added Appendix 5 Toxicity Scale 6. Updated the screening period from 12 weeks to 16 weeks
Protocol Amendment 2 (Version 3.0)	1. Clarification to Exclusion Criteria 1 and 3 (Hepatitis B and C language), 2 and 7. 2. Dose escalation criteria revised; added potential to include an intermediate dose. 3. Extended the screening period up to 12 weeks. 4. Streamlining study procedures to reduce participant burden. Revisions to study procedures, include: a. Removed spare plasma collections at Day 0, Weeks 1-11, 14-22, 30, 34, 46 b. Removed physical exam and weight collections at Weeks 16, 22, 30 and 46. c. Removed immunology collections at Screening, Day 0 post-FVIII and Day 0 post-vector and Day 1. d. Removed FVIII inhibitor collections at Weeks 7, 12, 14, 26 and 40 e. Removed FVIII dose at Week 52/EOS f. Clarified central lab vs. local lab collections 5. Added repeat of Week 46 procedures, except for ELISpot, in the event that a LTFU study is not enrolling at the time of the subject's Week 52/EOS visit. 6. Changed 'Hepatic transaminase elevation requiring immunosuppression' to a primary endpoint from a secondary endpoint. 7. Updated Section 5 to include all assessments for all study visits. 8. Clarified the Safety Reporting section including revisions to causality and severity assessments (Section 12.2), addition of new sections (i.e., Section 12.1.3 Adverse Events of Special Interest, Section 12.3 Time Period and Frequency for Collecting AE and/or SAE Information). 9. Removed the Independent Immunosuppressive Therapy Dosing Committee as none will be used in this study, 10. Clarified Section 13 Administrative Procedures and Section 13 Further Requirements and General Information, including addition of new sections

Document	Description of Main Changes
	(i.e., Section 13.3 Source Documents, Section 13.6 Study and Site Closure). 11. Additional wording updated throughout the protocol to provided clarity.
Protocol Amendment 1 (Version 2.0)	1. Increased number of participants from 18 to 30. 2. Revised Inclusion Criteria 3, 4 and 7, and removed Inclusion Criterion #5. 3. Revised Exclusion Criterion 8. 4. Added requirement that 6-week interval required at each dose level for the first two participants at each dose level. 5. Clarified the Dose Expansion and Escalation criteria. 6. Specified the time points collected for immune profiling. 7. Revised the guidelines to initiate corticosteroid therapy. 8. Modified the study stopping rules for hepatic involvement.
Original Protocol (Version 1.0)	Not Applicable