PROTOCOL: SHP648-101

TITLE:	An Open-Label, Multinational, Phase 1/2 Study of the Safety and Dose Escalation of SHP648, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing FIX Padua in Hemophilia B Subjects
SHORT TITLE:	A Phase 1/2 study of SHP648, an Adeno-Associated Viral Vector for Gene Transfer in Hemophilia B Subjects
STUDY PHASE:	Phase 1/2
ACRONYM:	SHP648-101
DRUG:	SHP648[i] (AAV8.ss-3xCRM8-TTR-FIX_R338Lopt)
IND NUMBER:	19233
EUDRACT NUMBER:	2018-004024-11
OTHER NUMBER:	*Not Applicable*
SPONSOR:	Baxalta US Inc.* 300 Shire Way, Lexington, MA 02421 AND Baxalta Innovations GmbH* Industriestrasse 67, A-1221 Vienna * Baxalta is now part of Shire. Shire is now part of Takeda
PRINCIPAL/COORDINATING INVESTIGATOR:	TBD
PROTOCOL HISTORY:	**Amendment 1.0, 21 FEB 2020** Replaces: Original Protocol, 13 May 2019

[i] Also known as TAK748

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature:	**Date:**
[redacted], MD	

Investigator's Acknowledgement

I have read this protocol for Study SHP648-101.

Title: An Open-Label, Multinational, Phase 1/2 Study of the Safety and Dose Escalation of SHP648, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing FIX Padua in Hemophilia B Subjects

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS PROTOCOL VERSION

Protocol SHP648-101: Amendment 1, 21 FEB 2020

Replaces: Protocol SHP648-101: Original Protocol: 13 May 2019

Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number 1.0	**Amendment Date** 21 FEB 2020	**Global**
Protocol Amendment Summary and Rationale: Changes made in Protocol SHP648-101 Amendment 1 address the need for a higher number of subjects to be screened, for extension to a new region and introduction of a 6-week observational period between dosing of the first and the second subject of each cohort to comply with requests from the US Food and Drug Administration (FDA) and several Regulatory Agencies un Europe.		
Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Minor grammatical and/or administrative changes and rewording for better readability have been made.	To improve the readability and/or clarity of the protocol	Throughout the document
Updated name of the Sponsor Signatory	To be consistent with organizational changes	Protocol Signature Page
Added IND number	To be consistent across study documentation	Protocol Cover Page
Updated study contact information	To be consistent with organizational changes	Emergency Contact Information
Increased planned number of subjects to be screened to 60 subjects	To address the need for a higher number of subjects to be screened due to a high rate of screen failure	Synopsis: Subjects planned for screening and throughout the protocol
Increased planned number of sites to be initiated to 30 sites	To address the need for a higher number of sites to be included	Synopsis: Site(s) and Region(s) and throughout the protocol
Included the United States as one of the participating regions	To extend the study to a new region	Synopsis: Site(s) and Region(s) Section 4.5
Changed the study period from 2019 – 2023 to 2019 - 2024	To be consistent across study documentation	Synopsis: Study period (planned)
Updated the Study Rationale	Per a request from the German Regulatory Agency, PEI	Synopsis: Study Rationale Section 2.3.
[redacted]		
Added description of the safety follow up duration.	To improve clarity	Synopsis: Methodology Section 4.4

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Replaced internal Dose Escalation Committee by external Data Monitoring Committee	As per a request from the Central Ethics Committee of the Medical University in Vienna, Austria (Ethikkommision Medizinische Universität Wien)	Synopsis: Dose Escalation Section 6.2.1.1. and throughout the document
Included an observational period of at least 6 weeks between the first three subjects in the first Cohort and first and second subjects in Cohort 2 and Cohort 3	As per a request from the US FDA and the German Regulatory Agency, PEI	Synopsis: Dose Escalation Section 6.2.1.1
Clarified the schedule of data review by the DMC in case of dose cohort extension	To exclude any ambiguity and for consistency with the DMC Charter	Synopsis: Dose Escalation Section 6.2.1.1
Updated options for DMC recommendation	To exclude any ambiguity for Dose Extension/Escalation	Synopsis: Dose Escalation Section 6.2.1.1 Section 6.2.2
Updated duration of the observation period for the DMC decision	For improved clarity and to exclude any ambiguity regarding the decision criteria for Dose Extension/Escalation	Synopsis: Dose Escalation Section 6.2.1.1
Revised Study Stopping Criterion #1 for Enrollment and Dose Escalation	For improved clarity on the risk of thrombosis	Synopsis: Stopping Criteria for Enrollment and Dose Escalation Section 6.2.1.2
Added Study Stopping Criterion #2 for Enrollment and Dose Escalation to indicate that enrollment and escalation to next dose cohort must be paused if at week 14 a loss of endogenous FIX activity level >75% of the highest FIX activity level measured after SHP648 administration occurs despite corticosteroid prophylaxis.	To exclude any ambiguity and clarify that a loss in gene expression impacting an efficacy endpoint is a criterion to pause enrollment/dose escalation	Synopsis: Stopping Criteria for Enrollment and Dose Escalation Section 6.2.1.2
Study Stopping Criterion #9: Added to pause enrollment of additional subjects if any drug-related event occurs in SHP648 treated subjects that is deemed to pose an unacceptable risk to subjects by the investigator or medical monitor after further evaluation.	To prevent further dosing of the subjects until drug-related event deemed to pose unacceptable risk is fully evaluated by the DMC.	Synopsis: Stopping Criteria for Enrollment and Dose Escalation Section 6.2.1.2

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Specified the possibility for subjects to resume FIX prophylaxis regimen after week 14 when gene therapy treatment is insufficient to control or prevent spontaneous bleeds	Per a request from the German Regulatory Agency, PEI.	Synopsis: FIX Utilization Section 6.6.3.1
Added section in **Glucocorticoid Use in the Study:** "*Notwithstanding the protocol language requiring that two subjects must first be eligible for reactive corticosteroid treatment, the Sponsor's Medical Monitor with the concurrence of the Investigator, may permit the use of prophylactic steroids in any subject based on all available scientific evidence at the time of subject enrollment. Such evidence may include the clinical outcomes of others AAV-based gene therapy studies as well as nonclinical relevant data that may emerge. The possible use of corticosteroid will be communicated to potential subjects as part of the ICF process"*	To exclude any ambiguity regarding the decision criteria to administer glucocorticoids during the study	Synopsis: Glucocorticoid Use in the Study Section 6.6.3.2
Revised the criteria for employing glucocorticoid treatment to also list the criteria for implementing prophylactic glucocorticoid treatment	For improved clarity and to exclude any ambiguity regarding the decision criteria to administer glucocorticoids during the study	Synopsis: Glucocorticoid Treatment Section 2.4.1 Section 6.6.3.2
Revised the guidance for prednisolone dosing regimen to decrease the corticosteroid regimen duration	To closer align with current guidelines for the management of autoimmune hepatitis	Synopsis: Glucocorticoid Treatment Section 6.6.3.2, Table 4
Revised Inclusion Criterion #3	Per a request from the French Regulatory Agency ANSM and the German Regulatory Authority, PEI	Synopsis: Inclusion Criteria Section 5.1
Revised Inclusion Criterion #4	For improved clarity and to exclude any ambiguity	Synopsis: Inclusion Criteria Section 5.1 Section 5.4.1 Appendix 4

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Rearranged the order of the exclusions criteria	For improved readability	Synopsis: Exclusion Criteria Section 5.2
Revised Exclusion Criterion #4 Added: "*Subjects whose laboratory assessments are* ≤ 1:10 *may be re-tested within the same Screening window and, if eligibility criterion is met on retest, may be enrolled after confirmation by the Sponsor's Medical Monitor*."	For improved clarity and to exclude any ambiguity	Synopsis: Exclusion Criteria Section 5.2
Revised Exclusion Criteria and numeration for Criteria #6, #7 #8, #9, #24, #30 (#8 became #7, #24 became #28, #26 became #30)	For improved clarity	Synopsis: Exclusion Criteria Section 5.2
Revised Exclusion Criterion #15 to add preexisting diagnoses indicative of significant underlying liver disease, and tests to determine liver fibrosis status	For improved clarity and to exclude any ambiguity	Synopsis: Exclusion Criteria Section 5.2
Divided the information in originally Exclusion Criterion #15 into Exclusion Criteria #15 to #18.	For improved readability	Synopsis: Exclusion Criteria Section 5.2
Revised the threshold value provided for albumin level as a marker of liver disease in Exclusion Criterion #17 from "*Albumin ≤ 3.5 g/dL* to ". "*Albumin below the central laboratory's lower limit of normal*" and albumin results are combined with FibroTest / FibroSURE with a result > 0.48	To increase specificity of the tests	Synopsis: Exclusion Criteria Section 5.2
Added "*Patients who do not consent to participate in SHP648 Extension Study will be followed according to local safety regulations*."	For improved clarity on maximum duration of subject participation in Study SHP648-101 and planned safety follow-up.	Synopsis: Maximum Duration of Subject Participation Section 4.4
Revised the primary endpoint to not only cover incidence but also severity of AEs	Per request from German Regulatory Agency (PEI)	Synopsis: Primary Endpoint Section 3.2., Table 3 Section 9.5.1

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Transferred all information regarding sample collection schedules from Table 1 to Table 2	To be consistent with the current template for phase 2-4 clinical study protocols	Section 1.3, Table 1 and Table 2
Added the sample blood collection schedule for transcriptome analysis and metabolomics, as well as related footnote “m”; adjusted footnote “c” accordingly	For consistency with assessment schedules and descriptions in Sections 8.1 and 8.2.	Section 1.3.2, Table 2
Rearranged order of laboratory assessments to group similar parameters	To improve the readability and clarity of the protocol	Section 1.3.2, Table 2
Shifted blood draw between week 4 and week 14 for transcriptome and metabolomics from the clinic to the lab visit	To align with the back-up sampling schedule and to decrease the blood volume demand during the clinic visits	Section 1.3.2, Table 2 Section 8.1 Section 8.2
Specified time of Liver Function Testing	For clarity and consistency in assessment schedules	Section 1.3.2, Table 2
Added exome testing to the list of clinical laboratory assessments	For consistency with assessment schedules and descriptions in Sections 8.1 and 8.2. and Appendix 5	Section 1.3.2, Table 2
[redacted]		
Added that back-up samples are to be collected also pre-infusion	To align with metabolomics schedule	Section 1.3.2, Table 2 Section 8.1.2
Added footnote “s”	To specify that whole exome sequencing, transcriptomics and metabolomics is optional	Section 1.3.2, Table 2
Updated current information on Hemophilia B	To provide most current information	Section 2
Inserted additional literature references	To accurately report literature sources	Section 2.3 Section 11

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Inserted a cross-reference to Section 5.4.1	For improved readability of the document	Section 2.4.
Revised language to further clarify the benefit/risk balance	Per a request from the German Regulatory Agency, PEI	Section 2.4.
Clarified the use of azathioprine/prednisone in subjects that do not tolerate corticosteroid therapy	For improved clarity and to exclude any ambiguity regarding the eligibility for combination treatment with azathioprine / prednisolone	Section 2.4.1 Section 6.6.3.2
Deleted the short summary of the stopping/pausing criteria and kept a crosslink reference to the full list of criteria	To exclude any ambiguity	Section 2.4.1
Added clarification regarding the use of immunomodulating drugs other than corticosteroids during the study period	Per a request from the German Regulatory Agency, PEI.	Section 6.6.3
Further clarified the language on the medications not permitted 30 days prior to therapy	Per a request from the German Regulatory Agency, PEI.	Section 6.6.4
Revised the reasons for discontinuation	Per a request from the German Regulatory Agency, PEI.	Section 7.2.
Revised wording regarding subject withdrawal.	Per a request from the German Regulatory Agency, PEI.	Section 7.3.
Specified that transcriptome and metabolomics analyses may be performed if needed and if patient has provided informed consent	For improved clarity and to exclude any ambiguity	Section 8.1
Clarified the composition of the coagulation panel and the additional coagulation tests to be performed	For improved clarity and consistency with information in Table 2	Section 8.2.3.4.1
Added specifics of FibroSURE test	In alignment with Table 2, footnote “l”	Section 8.2.3.4.1
Clarified the process for communication of *F9* gene mutation and HLA genotyping results	To avoid any ambiguity	Section 8.2.3.4.3

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Specified that the genetic tests listed are mandatory and that voluntary genetic tests are listed in Section 8.2.4.3; added specifics of HLA test.	In alignment with Table 2, footnote "k"	Section 8.2.3.4.3
Streamlined the description of immunogenicity assays to be used in the study.	To provide only the information in scope for this document	Section 8.2.3.4.4
Moved Additional Sample Collection from Section 8.2.3.4.1 to Section 8.2.3.4.6	To improve the readability and clarity of the protocol	Section 8.2.3.4.6
[redacted]		
Changed duration of sample storage in biorepositories to 5 years.	For consistency within protocol	Section 8.2.4.3 Appendix 5
Consolidated information of blood drawing and storage, previously in Section 8.2.3.4 and Section 8.2.3.5, in Section 8.2.5	To improve the readability and clarity of the protocol	Section 8.2.5
Moved paragraph describing patient diary from Section 8.2.4.4 to Section 8.2.6	To improve the readability and clarity of the protocol	Section 8.2.6
Inserted additional language to specify the source data from patient diaries that may be modified.	For improved clarity and to exclude any ambiguity	Appendix 1.4
Moved paragraph describing treatment for subjects requiring urgent or emergency surgery after SHP648 administration from Section 7.3 to Appendix 3.1	To improve the readability and clarity of the protocol	Appendix 3.1
Inserted additional language on follow-up and documentation of SAEs deemed related to the investigational drug	To fulfill a request by the safety board.	Appendix 3.5
Harmonized the general study stopping rules due to safety concerns with the pausing/stopping criteria specific to Study 201501	For consistency with the study design	Appendix 3.11

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Description of Each Change	**Rationale**	**Section(s) Affected by Change**
Revised guidance text	For consistency with Section 5.1 and Section 5.4.1	Appendix 4
Updated Protocol History Table	To comply with the current template for phase 2-4 clinical study protocols and provide most current information	Appendix 8

See Appendix 8 for protocol history, including all amendments.

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the "Shire Clinical Study Adverse Event Form for Serious Adverse Events (SAEs) and Non-serious Adverse Events (AEs) as Required by Protocol" within 24 hours to the Shire Global Drug Safety Department. The fax number and e-mail address are provided on the form (sent under separate cover). A copy of this form must also be sent to the Sponsor's Medical Monitor using the details below.

Fax: [redacted]
email: [redacted]

For protocol- or safety-related questions or concerns <u>during normal business hours</u> (8:00 am-5:00 pm, Eastern Time), the investigator must contact the medical monitor:

[redacted] MD, PhD
[redacted]
e-mail: [redacted]
Mobile: [redacted]

For protocol- or safety-related questions or concerns <u>outside of normal business hours</u>, the investigator must contact:

Fax: [redacted]
email: [redacted]

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product quality complaints or non-medical complaints to Shire within 24 hours. If requested, defective product(s) will be returned to the sponsor for inspection and analysis.

A product quality complaint includes any instances where there is an allegation or report relating to Shire licensed or investigational products, received in writing, electronically, or orally, which indicates an impact to a product's strength, identity, safety, purity, or quality, or which suggests that a product did not meet the criteria defined in the regulatory applications, licenses, or marketing authorizations for the product. Examples of investigational product quality complaints include, but are not limited to, the following:

Unit issues	• Capsule fill empty or overage • Bottle/vial fill shortage or overage • Capsule/tablet damaged/broken • Syringe/vial cracked/broken	• Syringe leakage • Missing components • Product discoloration • Device malfunction
Labeling	• Label missing • Leaflet or Instructions For Use (IFU) missing • Label illegible	• Incomplete, inaccurate, or misleading labeling • Lot number or serial number missing
Packaging	• Damaged packaging (e.g., secondary, primary, bag/pouch) • Tampered seals • Inadequate or faulty closure	• Missing components within package
Foreign material	• Contaminated product • Particulate in bottle/vial • Particulate in packaging	

Please report the product quality complaint using the "Product Complaint Data Collection Form" via the email address:

[redacted]

Telephone number (provided for reference if needed):

Shire, Lexington, MA (USA)

[redacted]

For instructions on reporting AEs related to product complaints, see Appendix 3.4.

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1. PROTOCOL SUMMARY

1.1 Synopsis

Protocol number: SHP648-101	**Drug:** SHP648 (AAV8.ss-3xCRM8-TTR-FIX_R338Lopt)
Title of the study: An Open-Label, Multinational, Phase 1/2 Study of the Safety and Dose Escalation of SHP648, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing FIX Padua in Hemophilia B Subjects	
Short title: A Phase 1/2 study of SHP648, an Adeno-Associated Viral Vector for Gene Transfer in Hemophilia B Subjects	
Study phase: Phase 1/2	
Number of subjects (total and per treatment arm): **Subjects planned for screening:** up to 60 **Subjects planned for enrollment:** Up to 21 in total, with approximately 2-7 subjects per dose cohort and up to 3 dose-cohorts	
Investigator(s): Multicenter study	
Site(s) and Region(s): Up to 30 sites in the USA and several countries in and around Europe are planned.	
Study period (planned): 2019 – 2024	**Clinical phase:** 1/2

Objectives:

Primary:

- Assess the safety of single, escalating, intravenous (IV) doses of SHP648.

Secondary:

- Evaluate plasma FIX levels before and after SHP648 infusion and study the relationship between change in FIX activity and SHP648 dose.
- Evaluate bleeding episodes post-SHP648 administration.
- Assess humoral and cellular immune responses to FIX and the viral capsid (cp).
- Determine the duration of SHP648 genomes present in bodily fluids.
- Compare the consumption of exogenous FIX before and after gene transfer

Exploratory:

[redacted]

Rationale:

SHP648 is a single-stranded (ss) adeno-associated viral (AAV) vector designed to deliver a functional copy of the human coagulation factor 9 (*F9)* Padua gene to the liver and drive endogenous hepatocyte-specific expression of the FIX protein in hemophilia B patients. There are currently no approved gene therapy products for patients with hemophilia B, who solely rely on infusions of exogenous FIX for disease management. The rationale for this study is 1) to evaluate the expression of the high specific activity Padua FIX variant driven by the transthyretin promoter and, a novel liver-specific cis-regulatory module (CRM8) enhancer element; 2) to determine the safety of SHP648 and the dose required to achieve FIX activity levels between 30-50% of normal in subjects with severe and moderately severe hemophilia B.

Investigational product, dose, and mode of administration:

- SHP648 (AAV8.ss-3xCRM8-TTR-FIX_R338Lopt)
- Up to 3 dose cohorts
- Single peripheral IV administration

Methodology:

This is a first-in-man, open label, multicenter, dose escalation study evaluating safety and FIX expression in up to a total of 21 adult hemophilia B subjects.

Subjects who provide signed informed consent will undergo screening for study participation which includes a physical exam, electrocardiogram (ECG), and laboratory assessments for baseline measurements and evaluation of pre-existing immunity to AAV8. Those meeting eligibility criteria will be sequentially assigned to a dose cohort. Each subject will be administered a single IV infusion of SHP648 on Day 0, and then followed for 12 months in the primary study (see Figure 1, Study Schematic Diagram). Subjects will be asked to be followed up for at least 4 more years in a separate safety extension study to monitor long-term effects.

Dose Escalation:

Up to 3 doses will be evaluated in this study:

- Cohort 1 dose:
  - 4.0×10^{11} vector genome (vg)/kg body weight (BW)
- Cohort 2 dose:
  - 2-fold escalation of Cohort 1 dose: 8.0×10^{11} vg/kg BW

    OR
  - 3-fold escalation of Cohort 1 dose: 1.2×10^{12} vg/kg BW
- Cohort 3 dose:
  - 2-fold escalation of Cohort 2 dose
    - 1.6×10^{12} vg/kg BW (if cohort 2 dose was 8.0×10^{11} vg/kg BW) or 2.4×10^{12} vg/kg BW (if cohort 2 dose was 1.2×10^{12} vg/kg BW)

      OR
  - 3-fold escalation of Cohort 2 dose
    - 2.4×10^{12} vg/kg BW (if cohort 2 dose was 8.0×10^{11} vg/kg BW) or 3.6×10^{12} vg/kg BW (if cohort 2 dose was 1.2×10^{12} vg/kg BW)

Initially, one subject will be dosed in Cohort 1, followed by an observational period of at least 6 weeks. If no safety concern is observed in the first dosed subject, a second subject will be dosed. For each additional dose cohort, dosing of the second subject will be scheduled 6 weeks after dosing of the first subject at the earliest. If Cohort 1 is expanded, up to 7 subjects may be dosed in Cohort 1.

Decisions pertaining to cohort expansion, dose escalation, number of subjects to be dosed, as well as the exact dose for subsequent Cohort 2 and Cohort 3 will be made based on the recommendation provided by an external Data Monitoring Committee (DMC). The DMC will review data including the Week 14 visit of the second dosed subject in Cohort 1. The observation period for the DMC data cut in expanded Cohort 1 and for Cohorts 2 and 3 may be adjusted to between 6 and 14 weeks based on Cohort 1 FIX expression peak data observed in the first two patients, as well as, other relevant study data and published literature.

After review of all safety and FIX activity data, the DMC will provide recommendations to pursue one of the following:

1. Escalate to the next dosing cohort
2. Increase the number of subjects in the current dosing cohort
3. Cap enrollment at the current dosing cohort and complete the study
4. Enroll additional subjects into a previous lower dose cohort in order to obtain additional safety information
5. Enroll subjects into a modified dosing cohort that is intermediate between those into which subjects have been previously enrolled.

Stopping Criteria for Enrollment and Dose Escalation:

Enrollment and escalation to next dose cohort will be paused until an independent DMC evaluates all available study data and makes a recommendation if any one of the following criteria are met:

1. Achievement of vector-derived FIX activity level ≥ 150% in any subject at any time while on the study due to potential risk of thrombosis. The level of FIX activity is measured frequently throughout the study. Study enrollment will be paused based on the local laboratory results (Table 1 and Table 2)
2. First two subjects on corticosteroid prophylaxis experience, at week 14, a loss of endogenous FIX activity level >75% of the highest FIX activity level measured after SHP648 administration (based on one stage central laboratory). The level of FIX activity is assessed frequently throughout the study (Table 1 and Table 2).
3. A value of 3 times the upper limit of normal (3 x ULN) or greater in ALT (alanine aminotransferase), AST (aspartate aminotransferase), or both in any subject after SHP648 administration that is not responsive after 12 weeks of corticosteroid rescue treatment.
4. A serious adverse event (SAE) that may be potentially related to SHP648 and which poses either an immediate risk to the subject's health or is likely to adversely affect the subject's health long term. This includes events classified as AEs qualifying for special notification (Brussels, 03/12/2009, ENTR/F/2/SF/dn D(2009) 35810; EMEA/CHMP/GTWP/60436/2007), if these are judged as potentially related to SHP648.
5. The development in any subject of an inhibitor towards FIX (or FIX Padua) after having received SHP648. Further investigations of the characteristics and potential contributing factors and causal relationships of the observed FIX (or FIX Padua) inhibitor will be initiated.

6. Death of a subject, after having received SHP648, that is judged as, definitely, probably or possibly attributed to SHP648. Enrollment in the study and further dosing will be temporarily stopped in order to undergo review by the applicable regulatory authorities and the DMC.
7. Occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to SHP648.
8. Occurrence of moderate or severe-drug-related AEs in 2 or more SHP648 treated subjects in a given cohort.
9. If any other drug-related event occurs in SHP648 treated subjects and is deemed to pose an unacceptable risk to subjects by the investigator or medical monitor after further evaluation, additional subjects will not be enrolled or dosed until a decision is taken to stop or proceed with the study based on further evaluation of the available data by the DMC. Following a safety review of the event, study enrollment or dosing of subjects in the screening period may be restarted if the medical monitor and the investigator determine that it is safe to proceed with the study.

Study enrollment or dosing of currently enrolled subjects may be restarted if the DMC considers that it is safe to proceed with the study. All the applicable rules regarding DMC decisions are addressed in the DMC Charter.

FIX Assessment

FIX activity levels will be measured by both the central laboratory (one-stage clotting and chromogenic assays) and the site's local laboratory (one-stage clotting or chromogenic assay) at all time points. However, only central laboratory FIX activity values derived from the one-stage clotting assay will be utilized for determining subject eligibility, establishing baseline FIX activity levels, and informing study dose escalation decisions. Local laboratory FIX activity values, in addition to central laboratory FIX activity levels, may be used to inform the initiation of corticosteroid treatment (described below under "Glucocorticoid Treatment in the Event of Hepatic Inflammation" below).

FIX Utilization

During the course of the study, utilization of exogenous FIX for the prevention (prophylactic treatment in case of FIX activity levels <2% and other cases when necessary at the discretion of investigator and medical monitor) or episodic management (on-demand treatment) of hemorrhages will be at the discretion of the Investigator and/or local hemophilia physician in consultation with the study subject. However, in order to measure FIX activity that is derived from the SHP648 transgene and not exogenous FIX products, subjects will be asked to refrain from prophylactic FIX usage between Weeks 3 to 14 post-SHP648 infusion and during the 3 weeks prior to Month 9. During these periods which are critical for the assessment of sustained vector-derived FIX expression (as both an efficacy and safety evaluation), on-demand treatment that is required for clinical bleeding should, if possible, be performed using a standard half-life FIX product rather than an extended half-life FIX concentrate. Whenever possible, a 5- day wash out period should be observed prior to any study visit with a FIX assessment, however, scheduled visits should not be missed in the case of a wash out of less than 5 days.

Prophylactic regimen with exogenous FIX may be resumed after Week 14 in case of FIX activity levels <2% and/or at the discretion of the Investigator and in consultation with the Sponsor's Medical Monitor, for subjects in whom gene therapy is insufficient to control or prevent spontaneous bleeds.

Glucocorticoid Use in the Study

Should two subjects in any dosing cohort experience hepatic inflammation requiring reactive glucocorticosteroid treatment, subjects who are subsequently dosed with SHP648 will receive prophylactic prednisolone (or prednisone) treatment starting at Day 8 after SHP648 infusion. Prophylactic treatment should follow the regimen proposed in the Prednisolone Dosing Regimen table provided below. The corticosteroid regimen may be adjusted at the discretion of the Investigator in consultation with the Sponsor's Medical Monitor depending on the subject's tolerance of the regimen and the observed hepatic transaminase response Patients receiving corticosteroids may require additional visits to evaluate potential side effects of corticosteroid therapy and for dose adjustments, FVIII activity and liver function tests (LFTs) will be measured at these visits by local laboratories.

Notwithstanding the protocol language requiring that two subjects must first be eligible for reactive corticosteroid treatment, the Sponsor's Medical Monitor with the concurrence of the investigator, may permit the use of prophylactic steroids in any subject based on all available scientific evidence at the time of subject enrollment. Such evidence may include the clinical outcomes of others AAV-based gene therapy studies as well as nonclinical relevant data that may emerge. The possible use of corticosteroid will be communicated to potential subjects as part of the ICF process.

Glucocorticoid Treatment

A tapering course of glucocorticosteroid treatment initiated with 60 mg/day of prednisolone (or prednisone) will be employed:

- **Reactively**, if any of the following criteria are met:
  a. A subject demonstrates an apparent vector-associated FIX expression of greater than 3-fold baseline activity level
  (e.g., > 3-6% FIX activity level) followed by a loss of > 66% of any confirmed FIX activity value over a 1- to 2-week period;

  AND/OR

  b. A subject demonstrates a clinically significant elevation in ALT defined as:
     - 1.5x baseline value obtained at Screening Visit

       OR

     - Above the ULN
- **Prophylactically** at Day 8 after SHP648 infusion, if 2 subjects from the same dosing cohort experience hepatic inflammation requiring reactive glucocorticosteroid treatment.

A tapering course of corticosteroids (prednisolone or prednisone depending on which product is approved in the region) will be given to interrupt any evidence of clinical or subclinical hepatic inflammation and to support the potential for sustained transgenic FIX expression. The following is provided as guidance for the dosing regimen with corticosteroids and must be tailored to the individual subject depending on the tolerance of the regimen and the observed hepatic transaminase response at the discretion of the Investigator and in consultation with the Sponsor's Medical Monitor.

Guidance for Prednisolone Dosing Regimen

Prednisolone Dose (mg/day)	Duration
60	1 week or more until AST/ALT decline is observed
40	1 week
30	1 week
25	1 week
20	1 week
15	1 week
12.5	2 weeks
10	2 weeks
5	1 week

Source: Terziroli Beretta-Piccoli et al., 2017
Note: Determine ALT and FIX activity weekly or twice weekly (as per study protocol).

Inclusion and Exclusion Criteria:

Inclusion Criteria:

1. Male, aged 18 to 75 years at the time of screening.
2. Established severe or moderately severe hemophilia B (plasma FIX activity ≤ 2% measured following ≥ 5 half-lives of most recent exposure to exogenous FIX) and either ≥ 3 hemorrhages per year requiring treatment with exogenous FIX or use of prophylactic therapy.
3. History of > 150 exposure days to exogenously administered FIX concentrates or cryoprecipitates.
4. Sexually active man must agree to use a condom during sexual intercourse or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners in the period from SHP648 administration until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least 2 consecutively collected semen samples assessed at the central laboratory (this criterion is applicable also for subjects who are surgically sterilized).
5. Signed informed consent

Exclusion Criteria:

1. Bleeding disorder(s) other than hemophilia B.
2. Documented laboratory evidence of having developed inhibitors (≥ 0.6 Bethesda Units [BUs] on any single test) to FIX proteins at any time.
3. Documented prior allergic reaction to any FIX product.
4. Anti-AAV8 neutralizing antibody titer > 1:5. Subjects whose laboratory assessments are ≤1:10 may be re-tested within the same Screening window and, if eligibility criterion is met on retest, may be enrolled after confirmation by the Sponsor's Medical Monitor.
5. Known hypersensitivity to prednisolone or prednisone, or to any of the excipients.
6. Having a disease in which treatment with prednisolone or prednisone is not tolerated (including, but not limited to osteoporosis with vertebral fractures, avascular necrosis, cataracts and glaucoma, difficult to control hypertension and diabetes as assessed by the treating physician).

7. Active Hepatitis C, as indicated by detectable Hepatitis C virus (HCV) ribonucleic acid (RNA) by reverse-transcriptase polymerase chain reaction (rtPCR).
8. Hepatitis B, as indicated by positive surface Hepatitis B virus (HBV) antigen test.
9. Evidence of markers of potential underlying risk for autoimmune mediated hepatic disease:
   a) Anti-smooth muscle antibody (ASMA) titer ≥ 1:40. Values of 1:31 to 1:39 will be flagged as possibly abnormal and the Investigator and Medical Monitor will evaluate the subject for eligibility.
   b) Elevated anti-liver-kidney microsomal antibody type 1 (LKM1) titers.
   c) Total IgG > 1.5x ULN.
   d) Antinuclear antibody (ANA) titer > 1:320 OR ANA titer > 1:80 if demonstrated concurrently with ALT that is > ULN.
10. Receiving chronic systemic antiviral and/or interferon therapy within 4 weeks prior to enrollment.
11. Clinically significant infections (e.g., systemic fungal infections) requiring systemic treatment.
12. Known immune disorder (including myeloma and lymphoma).
13. Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders.
14. An absolute neutrophil count < 1000 cells/mm^3.
15. History of liver biopsy or imaging indicating moderate or severe fibrosis (Metavir fibrosis stage F2 or greater).
16. History of ascites, varices, variceal hemorrhage, or hepatic encephalopathy.
17. Any of the following pre-existing diagnoses, which are indicative of significant underlying liver disease, are present in the medical record: portal hypertension, splenomegaly.
    a) A subject is not eligible if the serum albumin level is below the central laboratory's lower limit of normal;

       AND

    b) FibroTest/FibroSURE with a result > 0.48. Subjects with borderline Fibrosure assessments may be enrolled after confirmation by the Sponsor's Medical Monitor. Of note, if a subject has a known history of Gilbert's syndrome, A FibroTest cannot be used for fibrosis testing.
18. Markers of hepatic inflammation or cirrhosis as evidenced by 1 or more of the following:
    a) Platelet count < 150,000/µL.
    b) Total bilirubin > 1.5x ULN and direct bilirubin ≥ 0.5 mg/dL.
    c) ALT or AST > 1.0x ULN.
    d) Alkaline phosphatase > 2.0x ULN.
19. Prothrombin time international normalized ratio (INR) ≥ 1.4.
20. Serum creatinine > 1.5 mg/dL.
21. HIV if $CD4^+$ cell count ≤200 mm3 and/or viral load >20 copies/ milliliter (mL)
22. Urine protein > 30 mg/dL.
23. Body mass index > 38.
24. Major surgery planned within 6 months after enrollment.
25. Acute or chronic disease that, in the opinion of the Investigator, would adversely affect subject safety or compliance or interpretation of study results.

26. Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0.
27. Significant cardiovascular disease (such as New York Heart Association Class III or IV cardiac disease, congestive heart failure, myocardial infarction within the previous 6 months, unstable arrhythmias, or unstable angina) or significant pulmonary disease (including obstructive pulmonary disease).
28. History of arterial or venous thrombosis / thromboembolism, or a known pro-thrombotic condition.
29. Recent history of psychiatric illness or cognitive dysfunction (including drug or alcohol abuse) that, in the opinion of the Investigator, is likely to impair subject's ability to comply with protocol mandated procedures.
30. Concurrent enrollment in another clinical study, unless it is an observational (non-interventional) clinical study that does not interfere with the requirements of the current protocol or have the potential to impact the evaluation of safety and efficacy of SHP648 and with prior consultation with the Sponsor's Medical Monitor
31. Subject is family member or employee of the Investigator.

Maximum duration of subject participation in the study:

- Planned duration of screening period: up to 70 days prior to Day 0 for every subject
  - The planned enrollment period is about 45 days for each cohort to ensure sufficient subject enrollment
- Planned duration of treatment period: 1 day for single infusion
- Planned maximum duration of subject's participation approximately 15 months.
- Planned duration of follow-up after treatment: 12 months of monitoring post-SHP648 dosing in Study SHP648-101 followed by at least 4 years of long-term observation in a separate SHP648 Extension Study. Patients who do not consent to participate in SHP648 Extension Study will be followed according to local safety regulations.

Endpoints and Statistical analysis:

Analysis sets:

The Safety Analysis Set will consist of all subjects who receive any amount of investigational product (IP).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline FIX activity assessment.

Primary Endpoints

- Incidence and severity of AEs (serious or non-serious) related to IP that include development of FIX inhibitory antibodies, ECG findings, and clinically significant changes in standard laboratory parameters and in vital signs

Secondary Endpoints

- Circulating plasma FIX activity and FIX antigen levels
- Annualized bleed rate (ABR) in comparison to before gene transfer
- Neutralizing and binding antibody titers to AAV8
- T-cell response to AAV8 and FIX transgene products
- Presence of SHP648 genome by type of bodily fluid
- Percentage of change in consumption of exogenous FIX before and after gene transfer

Exploratory Endpoints

[redacted]

Statistical Analysis

All safety analyses will be performed using the Safety Analysis Set. All efficacy analysis will be performed over the FAS.

In general, descriptive summaries will be presented. Continuous variables will be summarized using mean, standard deviation, maximum, minimum, median, and other percentiles as appropriate. Categorical variables will be summarized using frequency counts and percentages. Analyses will be performed within each cohort separately and overall when appropriate.

Adverse events (AEs) will be coded using the MedDRA. Treatment-emergent AEs (TEAEs) are defined as AEs with start dates at the time of or following the first exposure to IP. The number of events and percentage of TEAEs and AEs related to IP will be tabulated by system organ class, by preferred term, and by treatment cohort. TEAEs and AEs related to IP will be further summarized by severity. AEs leading to withdrawal, SAEs and deaths will be listed.

Clinical laboratory tests, vital signs, and ECG findings will be summarized by treatment cohort and visit. Criteria for clinically significant changes in laboratory parameters will be specified in the statistical analysis plan (SAP), with number and percent of subjects with such changes tabulated by cohort.

Development of inhibitory and total binding antibodies to FIX and vector-derived FIX will be descriptively summarized.

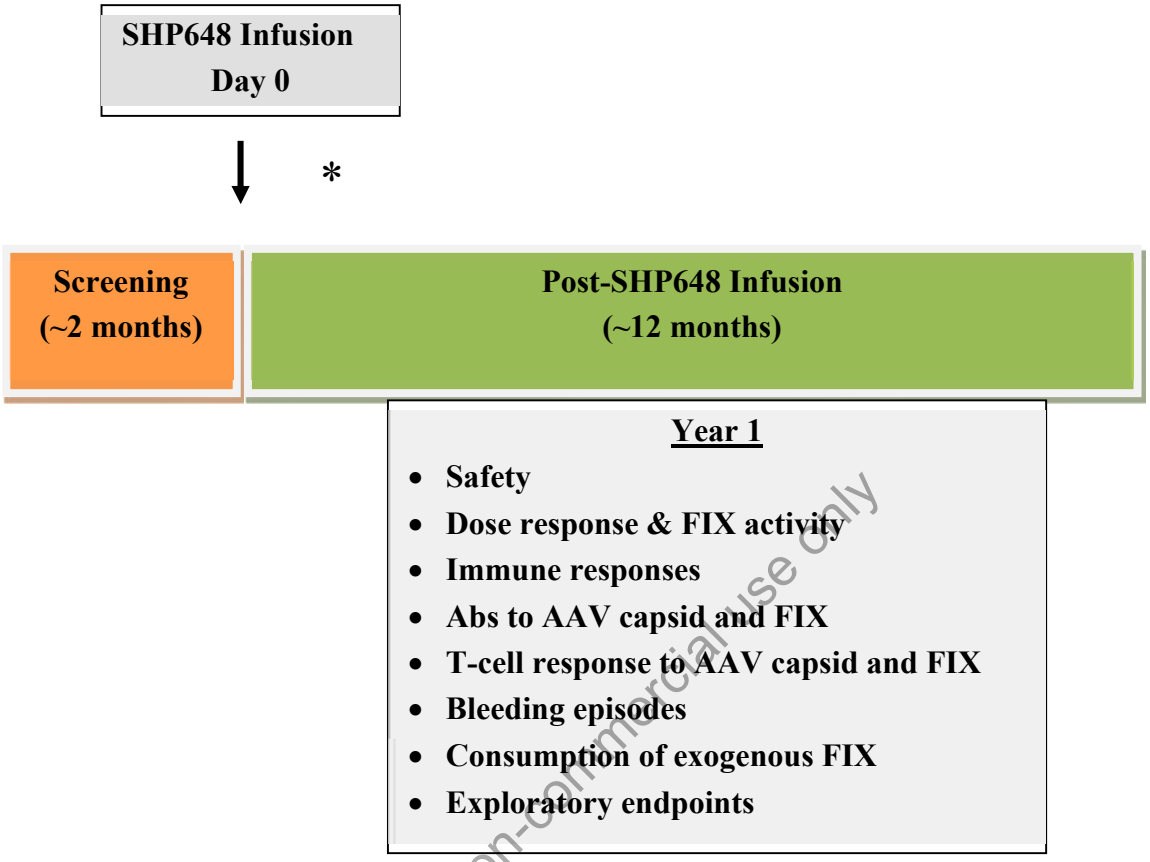
FIX activity and FIX antigen levels will be summarized by visit. ABR and exogenous FIX consumption data will also be descriptively summarized.

More detailed information about summarization of data, graphical representation, and analysis conventions will be provided in the SAP.

In addition to safety data reviews by the DMC, additional interim analyses on efficacy and safety data may be performed at study milestones (e.g., when the first two subjects in the first cohort finish the Week 14 visit, or when the first 2 subjects in the last cohort finish the Week 14 visit), or otherwise up to 2 times every year for the duration of the study. Analyses due to different trigger points may be waived or combined for efficiency.

1.2 Schema

Figure 1. Study Schematic Diagram



* Safety and FIX activity data through Week 14 from at least 2 dosed subjects in a cohort will be utilized to inform cohort expansion and dose escalation decisions.

Abbreviations: FIX = coagulation FIX, Ab = antibody, AAV = adeno-associated virus

1.3 Schedules of Study Activities and Assessments

1.3.1 Study Activities

Table 1. Schedule of Activities

Procedures	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0[b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**24 hours ± 6 hours after infusion**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
Informed consent[e]	X[e]									
Eligibility criteria review	X	X								
Demographic information	X									
Full physical examination including height and weight	X							X		X
Targeted physical examination		X	X	X	X	X	X		X	
Target joint evaluation	X							X		X
Medical history	X									
Laboratory assessments (see Table 2)	X	Pre- & post-infusion	X	X	X	X	X	X	X	X

Table 1. Schedule of Activities

Procedures	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0[b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**24 hours ± 6 hours after infusion**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
12-lead ECG[f]	X[f]		X[f]							
Vital signs	X	Pre- & post-infusion[g]	X	X	X	X	X	X	X	X
SHP648 treatment		X								
[redacted]										
AE review			←==========							→
Bleeding episodes			←==========							→
FIX utilization	X		←==========							→
Change in intensity of physical activity	X		←==========							→

Table 1. Schedule of Activities

Procedures	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0[b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**24 hours ± 6 hours after infusion**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
Concomitant medications	X		←==========							→[h]
Distribute/ review of patient diary[h]	X		←==========							→[h]

Abbreviations: FIX = coagulation factor IX; [redacted]; 12-lead ECG = 12-lead electrocardiogram; AE = adverse event; SHP648 = AAV-based FIX gene therapy product

a. If the Screening Visit occurs >70 days prior to Day 0 visit, then the Screening Visit assessments must be repeated.
b. At the discretion of the investigator, subjects will be monitored for the first 8 hours and may remain in the infusion center for 24 hours following infusion or return to the center for follow-up at 24 hours post-infusion (Day 1 Visit).
c. **Clinic Visits** include: all indicated laboratory assessments;
Lab Visits include: local laboratory Liver Function Test, FIX activity assessment, and backup sample collection ONLY.
d. In cases of withdrawn or discontinuation.
e. If the 5-day wash out period is part of the site's routine praxis, informed consent can be collected at the Screening Visit. Otherwise the informed consent must be collected beforehand to include a wash out period of 5 days as part of the study.
f. 12-lead ECG will be performed locally
g. Up to 3 hours pre-infusion and at 5, 15, and 30 mins and 1, 2, 3, 4, 6, and 8 hours post-infusion
h. To be reviewed at every scheduled and unscheduled study visit

1.3.2 Clinical Laboratory Assessments

Table 2. Clinical Laboratory Assessments

Laboratory Assessments	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0 [b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**N/A**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
Hematology[e]	X	X[f]	X	X	Weeks[g] 5, 7, 9, 11, & 13	X	X	X	X	X
Coagulation[h]	X	X[f]	X	X	Weeks[g] 5, 7, 9, 11, & 13	X	X	X	X	X
aPTT	X	Pre-infusion	X	X	X[q]	X	X	X	X	X
Clinical chemistries[i]	X	X[f]	X	X	Weeks[g] 5, 7, 9, 11, & 13	X	X	X	X	X
Liver Function Test[j] (Central and/or Local Laboratories)	X[g]				Central[g]: Weeks 4, 6, 8, 10, 12, & 14 Local : twice weekly at Clin and Lab					
FibroSURE[l]	X							X	X	X
[redacted]	X			Week 1	Week 6[g]			X		X

Table 2. Clinical Laboratory Assessments

Laboratory Assessments	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0 [b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**N/A**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
HIV serology	X									
Hepatitis B surface antigen	X									
Hepatitis C antibody	X									
HCV RNA	X			Week 1	Week 6[g]			X	X	X
Markers of autoimmune-mediated hepatitis[n]	X									
Serum cytokines[o]		X[f]	X							
MHC haplotype[k]	X									
FIX genotyping	X									
FIX activity (one-stage and chromogenic assays; central and local laboratories)[p]	X	Pre-infusion	X	X	X[q]	X	X	X	X	X

Table 2. Clinical Laboratory Assessments

Laboratory Assessments	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0 [b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**N/A**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
FIX antigen	X	Pre-infusion		X	X[g]	X	X	X	X	X
FIX inhibitor (Bethesda assay; central laboratory)	X			X	X[g]	X	X	X	X	X
Antibodies to FIX and FIX transgene product	X				Weeks[g] 6 & 8		Months 6 & 9	X	X	X
Neutralizing and binding antibodies to AAV8 and AAV2	X	Pre-infusion		Week 2	Weeks[g] 4, 8, & 12		X	X	X	X
PBMCs for CMI response to AAV8 and FIX transgene products	X			X	X[g]	X	Months 4, 5 & 6	X	X	X
Vector genome shedding via PCR in blood, saliva, urine, stool, and semen[r]	X		X	X[r]	X[g,r]	X[r]	X[r]	X		X

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Laboratory Assessments	Screening[a]	Treatment Period	Follow-Up Period Visits							
		Day 0 [b] (SHP648 Dose)	Day 1	Weeks 1-3 Once weekly (Clinic only)	Weeks 4-14 Twice weekly (Clinic & Lab)[c]	Week 15 Once weekly (Clinic only)	Months 4, 5, 6, 9 Once monthly (Clinic only)	Month 12 Study Completion Visit	Unscheduled Visit	Early Study Termination Visit[d]
Time Window	**Up to 70 days before Day 0 AND at least 5 days after last FIX dose**	**N/A**	**N/A**	**±1 day**	**±1 day for Clinic Visits Lab Visits: 3-4 days after Clinic Visit**	**±1 day**	**±4 days**	**±7 days**	**NA**	**NA**
Urine protein, total	X									
Backup samples	X	Pre- & 8h post-infusion	X	X	X[t]	X	X	X	X	X
Transcriptomics, and metabolomics[m,s]		Pre- & 8h post-infusion	X	X	X[t]	X	X	X	X	X
Exome testing[s]	X									

Abbreviations: aPPT = activated partial thromboplastin time; RBC = red blood cells, FIX = coagulation factor IX, BUN = blood urea nitrogen; ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl-transpeptidase, and LDH = lactic dehydrogenase, CK = creatine kinase; LFT = Liver function test, ANA = antinuclear antibody; PCR = polymerase chain reaction; HIV = human immunodeficiency virus ; MHC = major histocompatibility complex ; HCV RNA = hepatitis C virus ribonucleic acid ; AAV8 and AAV2 =adeno associated virus serotype 8 and serotype 2; PBMCs = peripheral blood mononuclear cells; CMI = cell-mediated immunity.

a. If the Screening Visit occurs >70 days prior to Day 0 visit, then the Screening Visit assessments must be repeated.

b. At the discretion of the investigator, subjects will be monitored for the first 8 hours and may remain in the infusion center for 24 hours following infusion or return to the center for follow-up at 24 hours post-infusion (Day 1 Visit).

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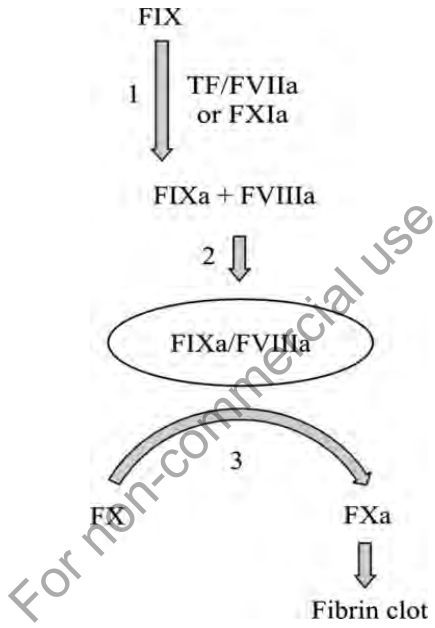
c. **Clinic Visits** include: all indicated laboratory assessments;
Lab Visits include: local laboratory Liver Function Test, FIX activity assessment, backup sample and voluntary whole blood collection for transcriptome ONLY.
d. In cases of withdrawn or discontinuation.
e. Hematology panel includes: complete blood count (hemoglobin, hematocrit, erythrocytes [i.e., RBC count], and leukocytes [i.e., white blood cell count]) with differential (i.e., basophils, eosinophils, lymphocytes, monocytes, neutrophils), platelet counts, RBC distribution width, mean corpuscular volume, and mean corpuscular hemoglobin concentration.
f Time points for Day 0 sample collection: (a) pre-infusion: up to 3 hours prior to SHP648 infusion, and (b) post-infusion: 30 mins (±5 mins), 4 hours (±10 mins), and 8 hours (±15 mins).
g. To be performed during **Clinic Visits** only, not during Lab Visits.
h. Coagulation panel includes: prothrombin time, international normalized ratio, thrombin time, D-dimer, fibrinogen activity.
i. Clinical chemistry panel includes: sodium, potassium, chloride, carbon dioxide, BUN, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, CK, bilirubin (total & direct), ALT, AST, ALP, GGT, and LDH.
j. Liver Function Tests (LFTs) include: ALT, AST, and GGT;
- Local laboratory LFTs performed at screening, then twice weekly between Weeks 4-14 (at **Clinic and Lab Visit**s).
- Central laboratory LFTs performed at Weeks 4, 6, 8, 10, 12, & 14, at Clinic Visits only.
k. 4-digit analysis of the following loci: HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRB1, and HLA-DRB3/4/5.
l. FibroSURE Score provided based on an algorithm that includes following laboratory results: ALT, alpha-2-macroglobulin, apolipoprotein A1, total bilirubin, gamma glutamyl transferase.
m. The collection of whole blood for transcriptome analysis on Day 0 will occur at the pre-infusion and 8 hours post infusion time points and the plasma/serum sample for the metabolomics analysis will be obtained from the back-up sample at the same timepoints as for transcriptome
n. ANA, Total IgG, anti-smooth muscle antibody, anti-LKM1 titers.
o. Serum cytokines including IL-6 and TNFα
p. All samples collected for FIX activity assessment will be measured at the central laboratory with the one-stage assay and with the chromogenic assay. FIX activity will also be assessed by the local laboratory at all of the indicated time points and at the **Clinic and Lab Visits** (Weeks 4-14). The local laboratory may use either the one-stage or the chromogenic FIX. activity assay, depending upon which assay is the standard in the individual local laboratory and provides the most rapid result; however, the same assay must be used consistently throughout the study.
q. To be performed weekly at the **Clinic and Lab Visits**
r. Vector shedding assessed at Screening, Day 1, weekly between Weeks 1-15 (at **Clinic Visits**), and at Months 4,5,6 & 9 as well as at Study Completion Visit (Month 12) until two consecutive negative results are obtained in the sample.
s. Whole exome sequencing, transcriptomics and metabolomics will be performed if subject provides consent
t. Backup sample and transcriptomic collection to be performed between Weeks 4-14 during Lab Visits only.

2. INTRODUCTION

Hemophilia B is a X-linked recessive bleeding disorder caused by mutations in the gene encoding clotting factor IX (FIX) that result in disruption of the normal clotting pathway. FIX is synthesized in the liver and circulates in the blood as a proenzyme. In the coagulation cascade, FIX is activated to FIXa by contact activation via factor XIa (FXIa) or as a consequence of vascular wall injury via tissue factor-FVIIa (TF/FVIIa) complex. FIXa forms a complex with activated factor VIII (FVIIIa), and this complex activates factor X (FX) to FXa, which ultimately leads to the formation of a stable fibrin clot, as depicted in Figure 2.

Figure 2. Role of FIX in the Clotting Cascade



Hemophilia B affects 1 in 20,000 male births. Disease severity correlates directly with the concentration of functional FIX protein in the plasma. Severe disease is characterized as having <1% of normal plasma levels of FIX (100% = 1 IU activity/mL or approximately 5000 ng protein/mL). Approximately 40% of all patients with hemophilia B have FIX activity levels lower than 1% which is associated with frequent soft tissue or intra-articular bleeds. Patients with moderate disease (1-5% FIX levels) may also require prophylaxis to treat traumatic and spontaneous bleeding. Chronic intra-articular bleeding leads to arthropathy with eventual joint degeneration, while intracranial and soft-tissue bleeds can be life threatening. Patients with mild disease maintain 5 to 40% of normal FIX levels. Patients with mild disease rarely have spontaneous bleeds, but they are at risk for trauma-induced bleeding (Schulman, 2012).

2.1 Indication and Current Treatment Options

2.1.1 Approved Therapies for Hemophilia B

Current treatment for hemophilia B is based on replacement of the deficient FIX with IV injections of recombinant FIX protein prophylactically or as needed to treat bleeding episodes. Factor IX replacement therapy has been shown to be effective in limiting bleeds and reducing mortality and morbidity (Löfqvist et al., 1997, Manco-Johnson et al., 1994). To prevent propagation of hemorrhage and extended morbidity, people with hemophilia and their families are taught to administer FIX concentrates at home at the first signs of bleeding. People with severe forms of the disease may need ongoing, preventive infusions (referred to as "prophylactic therapy" or "prophylaxis").

Although FIX replacement therapy has improved the quality of life and prolonged the life expectancy of patients with hemophilia, there are several significant limitations associated with this therapy (Buckner et al., 2018). Because it is not curative, patients remain at risk for life-threatening bleeding episodes and chronic joint damage. In addition, FIX replacement therapy is constrained by the high cost, limited availability in some regions, and also short half-life of the clotting factors, about 18-24 hours for standard FIX concentrates up to 86-115 hours for bioengineered recombinant FIX products with extended half-life (Oldenburg et al., 2018, Petrus et al., 2010, Roth et al., 2001, Santagostino et al., 2016).

Another important limitation of replacement therapy is that up to 2-3% of patients with hemophilia B develop neutralizing antibodies against the administered FIX protein (clinically referred to as inhibitors), which may make further replacement therapy ineffective and make bleeding episodes extremely difficult to manage (Fischer et al., 2015, Katz, 1996, Puetz et al., 2014). Depending on the specific genetic defect, the administered protein may present neoantigens to the immune system, inducing formation of antibodies or inhibitors (Fischer et al., 2015, Katz, 1996, Puetz et al., 2014).

2.2 Product Background and Clinical Information

The investigational product (IP) SHP648 is a gene therapy product engineered to induce the production of human factor FIX (FIX) protein in the liver cells of individuals with severe congenital FIX deficiency (hemophilia B) who currently rely on disease management with infusions of exogenous FIX. SHP648 is an AAV8-based vector designed to deliver a functional copy of the human *F9* Padua gene to the liver and drive endogenous hepatocyte-specific expression of the FIX-Padua protein, a naturally-occurring FIX variant carrying the R338L amino acid substitution (Simioni et al., 2009). This amino acid alteration causes a gain-of-function mutation, resulting in the expression of a FIX protein with increased specific activity compared to wild-type.

The wild type AAV upon which the recombinant AAV vector is based, is a non-pathogenic, replication defective dependoparvovirus, which is not associated with human disease. During the manufacture of recombinant AAV gene delivery vectors, all viral genes are removed such that the only protein-coding deoxyribonucleic acid (DNA) sequence that is delivered in the SHP648 vector is the *F9*-Padua gene along with DNA elements required for expression of the potentially therapeutic FIX Padua protein. The rationale for the choice of the serotype of 8 for the SHP648 capsid is that, among the naturally occurring AAV serotypes, AAV8 is particularly efficient at infecting and directing gene expression in the human liver, with minimal "off-target" gene expression in other tissues (e.g., in antigen presenting cells). In addition, SHP648 incorporates a hepatocyte specific transthyretin enhancer / promoter to further restrict FIX-Padua expression to the target liver tissue and three tandem copies of a novel liver-specific cis-regulatory module 8 (CRM8) element, to further enhance tissue-specific gene expression.

SHP648 is intended to be given as a single one-time IV infusion by a healthcare provider, at a qualified Center. If successful transduction of hepatocytes is achieved, long-term hepatic expression and secretion of endogenous FIX into the blood stream may support hemostasis and in turn eliminate or reduce the prophylactic and/or on-demand use of exogenous FIX concentrate therapy in hemophilia B patients. In this initial Phase 1/2 clinical study up to 3 SHP648 doses will be investigated. A single dose based on observed therapeutic durability is to be determined from this Phase 1/2 study for further evaluation in future clinical studies.

See Section 6.1.1 for further information on SHP648 and its usage in this study.

Nonclinical dose response and toxicology studies with SHP648 have been completed. Data from these and additional nonclinical studies as well as a detailed description of SHP648 can be found in the SHP648 Investigator's Brochure (IB). No prior study of SHP648 in humans has been performed.

2.3 Study Rationale

Results from in-human FIX gene therapy studies have demonstrated that therapeutic levels of functional FIX can be achieved in a dose dependent manner using AAV-based vectors, leading to amelioration of the disease phenotype and reduction or even discontinuation of FIX replacement therapy (George et al., 2017, Manno et al., 2006, Miesbach et al., 2018, Monahan et al., 2015b, Nathwani et al., 2014, Nathwani et al., 2011). However, the efficiency of vector transduction is hindered in patients with pre-existing neutralizing antibody to the AAV capsid. Furthermore, in some patients the duration of transgene expression may be limited by induction of a cell-mediated immune response to the AAV capsid which manifests itself clinically by a transient elevation of liver transaminases (Manno et al., 2006, Miesbach et al., 2018).

More recent trials have excluded subjects with neutralizing antibodies to the AAV capsid and have used gene therapy vectors based on AAV serotypes for which humans have reduced pre-existing immunity and with preferential hepatic tropism (e.g., AAV8, AAV5; (George et al., 2017, Miesbach et al., 2018, Monahan et al., 2015b, Nathwani et al., 2014, Nathwani et al., 2011)). AAV vectors with superior hepatic tropism can be administered systemically, which is less invasive than intrahepatic infusion. Current approaches aim to develop FIX gene therapy products that could potentially reduce the dose of vector required to achieve effective levels of FIX activity, thereby reducing the risk of vector-related hepatic inflammation (Arruda et al., 2018, Mingozzi and High, 2017).

SHP648 vector is based on the AAV8 technology and utilizes the liver-specific transthyretin (TTR) enhancer-promoter in combination with 3 copies of the liver-specific CRM8 element (Chuah et al., 2014, Nair et al., 2014) to further enhance hepatic expression. In addition, the codon-optimization of the human *F9* transgene encoding a naturally-occurring variant of the coagulation FIX (FIX Padua, FIX_R338Lopt) protein has been revised resulting in a 5' cytosine – phosphate – guanine 3' (CpG)-motif depleted (and thus potentially less immunogenic) nucleotide sequence, that nonetheless confers increased expression compared to the wild-type nucleotide sequence. Finally, the vector design has been changed from self-complementary to single-stranded to further reduce the potential immunogenicity of the vector.

There are currently no approved gene therapy products for patients with hemophilia B, who solely rely on infusions of exogenous FIX for disease management. The rationale for this study is 1) to evaluate the expression of the high specific activity Padua FIX variant driven by the transthyretin promoter and, a novel liver-specific cis-regulatory module (CRM8) enhancer element; 2) to determine the safety of SHP648 and the dose required to achieve FIX activity levels between 30-50% of normal in subjects with severe and moderately severe hemophilia B.

2.4 Benefit/Risk Assessment of FIX Gene Therapy

Hemophilia B is a prime target disease for gene therapy, as it is caused by a single gene defect and has a broad therapeutic window. Furthermore, the *F9* transcript has a suitable size for the DNA-cargo capacity of AAV vectors (Miesbach and Sawyer, 2018, Nathwani et al., 2004, Petrus et al., 2010). Since even a modest increase in the level of missing FIX can ameliorate the severe bleeding phenotype, it is anticipated that the successful development of a hemophilia B gene therapy product will lead to continuous endogenous expression of FIX and thereby address many of the current treatment limitations by achieving sustained greater than 1% circulating FIX activity at all times. Adherence to repeated venipuncture and infusions would not be needed to achieve this clinical goal if gene transfer is successful, wherein the goal is for the disease to be cured with a single intervention that effects durable gene expression.

The investigational product SHP648 has not been tested in humans. The primary objective of the current study is to evaluate safety and to determine a dose of SHP648 that may lead to levels of FIX expression associated with freedom from spontaneous joint hemorrhage. As with all Phase 1 trials, there is no expectation that study subjects will receive direct benefit. However, based on modelling of data from preclinical studies, a starting dose from Cohort 1 was chosen with the possibility that it may provide a clinically meaningful FIX level. SHP648 doses for the current Phase 1/2 trial have been chosen based upon preclinical research suggesting that all of the chosen doses may lead to FIX expression; however, there is no prior experience with SHP648 in humans. For this same reason, if a single injection of SHP648 directs FIX expression in humans, the anticipated length of persistence of expression following a single injection of SHP648 in humans is currently not known.

A dose of SHP648 could potentially result in a higher level of FIX activity than desired. As FIX activity increases beyond 100% of normal values, beginning at levels of approximately 150%, the blood may clot abnormally (a condition called hypercoagulation). The resultant clots or thrombin may lead to ischemia in one or more organs or to disseminated intravascular coagulation, a life- threatening condition. The risk of hypercoagulability is managed in the study through careful dose selection, cautious dose escalation and patient staggering and review of all available data by an external independent Data Monitoring Committee (DMC) prior to dose escalations. In the unlikely event that sustained supernormal elevations of FIX levels are observed in a study subject, this can be managed through the use of standard anticoagulant agents. Evaluation of Anticipated benefits and Risks of Using an AAV-based Gene Therapy Product

The AAV8 vector is predominantly a non-integrating virus, and so there is a potential for the loss of the episomally maintained AAV vector genomes with division of the transduced cells, which would result in a loss of expression of the therapeutic transgene over time. Targeting clotting factor expression to post-mitotic hepatocytes with a low cellular turnover rate may minimize dilutional effects resulting from new hepatocyte generation (Kattenhorn et al., 2016). AAV8 vectors expressing clotting factors have led to expression for greater than 10 years (and counting) in large animals and greater than 6 years (and counting) in one human trial (Nathwani A.C. et al., 2018, Nathwani et al., 2014, Nathwani et al., 2011).

The long-term safety of recombinant AAV (rAAV) vectors in humans is unknown; however, AAV vectors have been delivered to several hundred human subjects to date, in trials for cystic fibrosis, α1-antitrypsin deficiency, rheumatoid arthritis, congestive heart failure, lipoprotein lipase deficiency, as well as hemophilia, and have been remarkably free of vector-related AE (Chapin and Monahan, 2018, Colella et al., 2018). Several clinical trials have used naturally occurring or engineered AAV serotype vectors to achieve expression of FVIII or Factor IX

(FIX). The chief risk that has been observed in human clinical trials using systemic delivery of AAV vectors is that the subject's immune system recognizes the AAV as an infectious agent and raises humoral (antibody-mediated) and potentially cell-mediated immune responses against the vector.

Wild-type AAV (WT AAV), the parent virus upon which rAAV vectors are based, is non-pathogenic, resulting in a milder immune response than would be expected for a pathogenic virus. In addition, WT AAV is replication-defective and cannot produce an active infection without the coincident infection of a helper virus. Recombinant AAVs are further rendered non-pathogenic by having all of the gene sequences of WT AAV removed, resulting in extremely low environmental risk related to rAAV viral shedding (Baldo et al., 2013). The risk of vertical transmission is addressed in Section 5.4.1.

Nevertheless, at doses of rAAV gene therapy vectors used clinically, it is anticipated that all subjects will develop neutralizing antibodies against the capsid serotype. Although development of AAV-specific neutralizing antibodies is not associated with any symptoms or AEs, these antibodies would persist for years and prevent the repeat treatment with the same AAV vector until they are naturally cleared. Furthermore, the AAV serotype-specific neutralizing antibodies may or may not also cross-neutralize rAAV made using alternative AAV serotypes and diminish their efficiency. This consideration is relevant since the expected persistence of expression from a single injection of SHP648 is currently unknown.

In addition to the humoral response described above, a clinical laboratory complication of asymptomatic liver transaminase elevation (in particular the relatively liver-specific ALT) has been documented in some subjects on multiple hemophilia gene therapy and other AAV liver-directed gene therapy trials (Manno et al., 2006, Miesbach et al., 2018, Nathwani et al., 2014). The systemic delivery of rAAV has been associated with a T lymphocyte response directed against the rAAV capsid that in many but not all cases has occurred coincident with the ALT elevations. This response has been ascribed to recall AAV-specific memory T cells previously generated following WT AAV natural infection in combination with a helper virus. Specifically, partial or complete loss of FIX expression following initial successful gene transfer has been observed in previous human clinical studies (Manno et al., 2006, Miesbach et al., 2018, Nathwani et al., 2014) utilizing liver-directed AAV vectors to correct hemophilia B. The loss of FIX expression could be demonstrated in some subjects to be associated with the expansion of AAV capsid-specific T effector lymphocytes in the peripheral blood, resulting in inflammatory signaling targeting the AAV transduced hepatocytes. This has been further noted clinically by observations of vector dose-dependent elevations of liver transaminase (serum ALT), which in all cases were within normal range and were asymptomatic.

It remains unknown whether the capsid-directed immune response is the initiating phenomenon that leads to the loss of gene expression or whether other mechanisms are primary with the T cell signaling being a secondary phenomenon. In several of the clinical studies, the observed transaminitis and/or decrease in FIX levels was treated with weeks to months of high, tapering dose corticosteroids (prednisone / prednisolone), which have a broad but not T-cell specific immunosuppressive action. Corticosteroid therapy has in general been associated with normalizing liver transaminase levels, and in some (although not all) trials, with maintenance of some level of clotting factor activity.

Besides the primary T-cell mediated immune responses, innate immune responses to rAAV infection have been described in the preclinical setting. However, such responses have rarely been detected systemically in human clinical studies.

Neutralizing antibodies ("inhibitors") to FIX or to FVIII have not developed in any AAV-directed trial of gene therapy for hemophilia (George et al., 2017, Manno et al., 2006, Miesbach et al., 2018, Nathwani et al., 2014, Rangarajan et al., 2017). The specific concern that expression of FIX after AAV gene transfer to hepatocytes might promote cellular stress via activation of the unfolded protein response (UPR) has recently been examined in a preclinical model, and a dose-dependent UPR could not be demonstrated, nor any negative effect upon liver pathology or FVIII immunogenicity (Zolotukhin et al., 2016). Moreover, data from large and small animal disease models suggests that liver-mediated expression of potentially immunogenic antigens, including FIX Padua variant protein, in the context of gene transfer, can mitigate the immune response to the transgenic protein, mediated in part by the induction of $CD4^+CD25^+FoxP3^+$ regulatory T-cells (Crudele et al., 2015, Perrin et al., 2016).

AAV is a single-stranded, replication-deficient DNA parvovirus. The life cycle of the wild type AAV includes the potential to exist in a latent state following integration into the host cell genome. Integration of the wild type AAV is facilitated by the AAV replication (rep) proteins, encoded by the AAV rep genes. All coding sequences of the wild type AAV (including the sequences encoding AAV rep genes) are deleted during construction of rAAV expression cassettes and rAAV vectors.

2.4.1 Evaluation of Anticipated Benefits and Risks of Using Prednisolone for Management of Presumptive Vector-Related Hepatitis

Vector-derived factor expression that is followed by a decrease in factor levels has been observed in previous hemophilia gene therapy trials (Manno et al., 2006, Monahan et al., 2015a). The factor level decrease has been attributed to an immune response directed against the successfully transduced cells. The SHP648 vector design incorporates a single-stranded genome structure and an efficient codon optimization which greatly reduces the content of CpG repeats. These features minimize the appearance of Pathogen Associated Molecular Patterns (PAMPs) so as to minimize potential innate immune signaling via Toll-like Receptors.

However, a tapering course of glucocorticosteroid treatment initiated with 60 mg/day of prednisolone (or prednisone) will be employed:

- **Reactively**, if any of the following criteria are met:
  a. A subject demonstrates an apparent vector-associated FIX expression of greater than 3-fold baseline activity level
  (e.g., > 3-6% FIX activity level) followed by a loss of > 66% of any confirmed FIX activity value over a 1 to 2-week period;

  AND/OR

  b. A subject demonstrates a clinically significant elevation in ALT defined as:
     - 1.5x baseline value obtained at Screening Visit

       OR

     - Above the ULN
- **Prophylactically** at Day 8 after SHP648 infusion, if 2 subjects from the same dosing cohort experience hepatic inflammation requiring reactive glucocorticosteroid treatment.

For additional details and guidance on glucocorticoid treatment in Study SHP648-101 refer to Section 6.6.3.2.

The rationale to use corticosteroids in an attempt to interrupt the course of the apparent cytotoxic T-cell response (CTL) response includes: 1) to maintain the health of the gene-transduced liver and 2) to rescue transgenic clotting factor expression, with the understanding that rescue (if possible) of even modest amounts of sustained circulating factor activity is expected to improve the severe bleeding phenotype AND that repeat administration of the same gene therapy vector will not be possible due to expected development of high titer and long-lived AAV neutralizing antibodies. The rAAV-associated liver inflammation has in most individuals resolved rapidly (i.e. within 2 weeks) with the institution of high dose corticosteroid therapy, followed by taper over 8-12 weeks (George et al., 2017, Miesbach et al., 2018, Nathwani et al., 2014). Of note, based on data from three different AAV hemophilia B gene therapy trials, the response to supportive corticosteroids appears to be highly variable: sustained clotting factor expression has varied from 0% to 100% in individuals who received supportive corticosteroids in these trials (George et al., 2017, Miesbach et al., 2018, Nathwani et al., 2014).

Utilization of supportive corticosteroid treatment to interrupt ongoing transaminitis/loss of factor activity involves an initial systemic high dose of prednisolone followed by a conservative taper over 5 to 8 weeks, patterned after the guidelines of the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL) for the treatment of autoimmune hepatitis (Manns et al., 2010, Nathwani et al., 2014, Soloway et al., 1972).

Chronic use of systemic steroids at doses of ≥5 mg daily is associated with a wide variety of AEs, although the risk for many of these AEs increases particularly with continuous use for greater than 3-6 months. Should subjects receive a course of prednisolone therapy of <3 months (per protocol) the most important risks include: low bone mineral density (osteoporosis), osteonecrosis, adrenal suppression, hyperglycemia and diabetes; sleep and psychiatric disturbances. Loss of bone mineral density may occur with the planned doses, although in a meta-analysis of adult patients, the fracture risk rose with duration of therapy of 3-6 months or longer (Liu et al., 2013, van Staa et al., 2002). Nevertheless, low bone mineral density and osteoporosis at baseline are prevalent in the hemophilia population, and understanding this risk is important. Osteonecrosis (most commonly at the femoral head) is greatest with courses longer than proposed in this protocol but has occurred with short-term glucocorticoid exposure. Adrenal suppression is expected after two weeks of prednisolone therapy (Liu et al., 2013) The protocol's empiric prednisolone taper minimizes this risk, and screening for this outcome will be performed following tapering of prednisolone to a physiologic dose (Ahmet et al., 2011, Liu et al., 2013). Although weight gain during the proposed course (<3 months) should be small, the development of Cushingoid features within 2 months of start of therapy is common (Fardet et al., 2007). Hyperglycemia may occur thus, glucose will be assayed regularly in all study subjects. Glucocorticoids use for even short periods of time may lead to psychiatric and cognitive disturbances including memory impairment, irritability, mood lability and sleep disturbance. If a subject does not tolerate corticosteroid therapy, Study Protocol SHP648-101 specifies the use of combination therapy with azathioprine/prednisone per the AASLD guidelines which allows lower cumulative glucocorticoid dose and risk.

The risks of the study will be managed by an external independent DMC based on pre-specified pausing/stopping criteria (refer to Section 6.2.1 for a full list of stopping/pausing criteria). A charter for the DMC will be separately provided.

Always refer to the latest version of the SHP648 investigator's brochure (IB) for the overall benefit/risk assessment and the most accurate and current information regarding drug metabolism, pharmacokinetics, efficacy, and safety of SHP648.

2.5 Compliance Statement

This study will be conducted in accordance with this protocol, the International Council for Harmonisation Guideline for Good Clinical Practice E6 (Integrated Addendum to ICH E6[R1]: Guideline for Good Clinical Practice E6[R2] Current Step 4 version, 9 November 2016), Title 21 of the US Code of Federal Regulations (US CFR), the EU Directives (2001/20/EC; 2005/28/EC), and applicable national and local regulatory requirements.

The responsibilities of the study sponsor and investigator(s) are described fully in Appendix 1.

3. OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objective

The primary objective of the study is to assess the safety of single, escalating, IV doses of SHP648.

3.1.2 Secondary Objectives

The secondary objectives of the study are as follows:

- Evaluate plasma FIX levels before and after SHP648 infusion and study the relationship between change in FIX activity and SHP648 dose.
- Evaluate bleeding episodes post SHP648 administration.
- Assess humoral and cellular immune responses to FIX and the viral capsid.
- Determine the duration of SHP648 genomes present in bodily fluids.
- Compare the consumption of exogenous FIX before and after gene transfer

3.1.3 Exploratory Objectives

[redacted]

3.2 Study Endpoints

A summary of endpoints supporting the study objectives is presented in Table 3.

Refer to Section 9 for a detailed description of endpoints and the planned statistical analysis.

Table 3. Objectives and Endpoints

Objective	Endpoint(s)
Primary	
• Assess the safety of single, escalating, IV doses of SHP648.	• Incidence and severity of AEs (serious or non-serious) related to IP that include development of FIX inhibitory antibodies, ECG findings, and clinically significant changes in standard laboratory parameters and in vital signs
Secondary	
• Evaluate plasma FIX levels before and after SHP648 infusion and study the relationship between change in FIX activity and SHP648 dose.	• Circulating plasma FIX activity and FIX antigen levels
• Evaluate bleeding episodes post SHP648 administration.	• ABR in comparison to before gene transfer
• Assess humoral and cellular immune responses to FIX and the viral capsid.	• Neutralizing and binding antibody titers to AAV8 • T-cell response to AAV8 and FIX transgene products
• Determine the duration of SHP648 genomes present in bodily fluids.	• Presence of SHP648 genome by type of bodily fluid
• Compare the consumption of exogenous FIX before and after gene transfer	• Percentage of change in consumption of exogenous FIX before and after gene transfer
Exploratory	
[redacted]	

4. STUDY DESIGN

4.1 Overall Design

Up to 21 adult male subjects with severe or moderately severe hemophilia B (FIX activity levels ≤ 2%) will be dosed in this open-label, multinational, safety and dose escalation study. Subjects will be administered a single IV infusion of SHP648 in one of three ascending dose cohorts, and monitored for safety, FIX expression, and immune responses for 12 months post-SHP648 infusion.

4.2 Scientific Rationale for Study Design

This study design will allow the study Sponsor and Regulatory Agencies to evaluate the safety and efficacy of an AAV8 Vector Expressing FIX Padua in Hemophilia B subjects as well as inform dosing decisions for a potential Phase 3 pivotal study. Safety and study progress will be monitored with a DMC that will provide a recommendation on further cohort size increase to collect additional data and dose modification based on data from previous cohorts.

4.3 Justification for Dose

SHP648-101 is to be initiated at a dose of 4.0×10^{11} vg/kg, which is expected to induce a FIX activity of 22% (according to results from nonclinical studies in mice) to 24% (according to results from nonclinical studies in rhesus monkeys). This clinical starting dose is 9.5 times lower than the "no observed adverse effect level" (NOAEL) of 3.8×10^{12} vg/kg (based on clinical release assay) determined during SHP648 non-clinical development. In term of AAV8 particle dose, the anticipated clinical starting dose (4.0×10^{11} vg/kg) is expected to reflect a particle titer dose of 5.5×10^{11} cp/kg. This represents an approximately 90-fold lower dose than the NOAEL for AAV 8 capsid particles (5.0×10^{13} cp/kg) determined during SHP648 non-clinical development. Similar to other gene therapy products, SHP648 is intended for single use only. Due to the demonstrated hepatic tropism of SHP648, it is to be administered by IV infusion (for further details refer to SHP648 Investigator's Brochure).

A DMC is included in the study design to provide ongoing monitoring of safety and study progress. At each dose level, a decision must be made regarding the safety of increasing to a higher dose level. The DMC will provide a recommendation on whether to increase the number of subjects at a dose level to obtain additional safety information, cap enrollment at a dose level, or modify a dose at a subsequent dose level, taking into consideration data that are observed in other cohorts.

4.4 Duration of Subject Participation and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 15 months. Study SHP648-101 will last maximum 48 months from First Subject First Visit until Last Subject Last Visit. Afterwards all patients will be invited to participate in a separate safety extension study (SHP648 Extension Study) for an observation period of at least 4 more years to monitor long-term effects. Patients who do not consent to participate in SHP648 Extension Study will be followed according to local safety regulations.

The Study Completion Date is defined as the date on which the last subject in the study completes the final protocol-defined assessment(s). This includes the follow-up visit or contact, whichever is later (refer to Section 8.1.3 for the defined follow-up period for this protocol).

4.5 Sites and Regions

Up to 60 subjects are planned to be screened for a total of up to 21 subjects enrolled. It is planned to have 2-7 subjects per dose cohort and up to 3 dose-cohorts.

Approximately 30 sites will be asked to participate, which will be located in the USA and several countries in and around the European region.

5. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

5.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. Male, aged 18 to 75 years at the time of screening.
2. Established severe or moderately severe hemophilia B (plasma FIX activity ≤ 2% measured following ≥ 5 half-lives of most recent exposure to exogenous FIX) and either ≥ 3 hemorrhages per year requiring treatment with exogenous FIX or use of prophylactic therapy.
3. History of > 150 exposure days to exogenously administered FIX concentrates or cryoprecipitates.
4. Sexually active man must agree to use a condom during sexual intercourse or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners in the period from SHP648 administration until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least 2 consecutively collected semen samples assessed at the central laboratory (this criterion is applicable also for subjects who are surgically sterilized).
5. Signed informed consent

5.2 Exclusion Criteria

The subject will be excluded from the study if any of the following exclusion criteria are met.

1. Bleeding disorder(s) other than hemophilia B.
2. Documented laboratory evidence of having developed inhibitors (≥ 0.6 BU on any single test) to FIX proteins at any time.
3. Documented prior allergic reaction to any FIX product.
4. Anti-AAV8 neutralizing antibody titer > 1:5. Subjects whose laboratory assessments are ≤ 1:10 may be re-tested within the same Screening window and, if eligibility criterion is met on retest, may be enrolled after confirmation by the Sponsor's Medical Monitor.
5. Known hypersensitivity to prednisolone or prednisone, or to any of the excipients.
6. Having a disease in which treatment with prednisolone or prednisone is not tolerated (including, but not limited to osteoporosis with vertebral fractures, avascular necrosis, cataracts and glaucoma, difficult to control hypertension and diabetes as assessed by the treating physician).

7. Active Hepatitis C, as indicated by detectable Hepatitis C virus (HCV) ribonucleic acid (RNA) by reverse-transcriptase polymerase chain reaction (rtPCR).
8. Hepatitis B, as indicated by positive surface Hepatitis B virus (HBV) antigen test.
9. Evidence of markers of potential underlying risk for autoimmune mediated hepatic disease:
    a. Anti-smooth muscle antibody (ASMA) titer ≥ 1:40. Values of 1:31 to 1:39 will be flagged as possibly abnormal and the Investigator and Medical Monitor will evaluate the subject for eligibility.
    b. Elevated anti-liver-kidney microsomal antibody type 1 (LKM1) titers.
    c. Total IgG > 1.5x ULN.
    d. Antinuclear antibody (ANA) titer > 1:320 OR ANA titer > 1:80 if demonstrated concurrently with ALT that is > ULN.
10. Receiving chronic systemic antiviral and/or interferon therapy within 4 weeks prior to enrollment.
11. Clinically significant infections (e.g., systemic fungal infections) requiring systemic treatment.
12. Known immune disorder (including myeloma and lymphoma).
13. Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders.
14. An absolute neutrophil count < 1000 cells/mm^3.
15. History of liver biopsy or imaging indicating moderate or severe fibrosis (Metavir fibrosis stage F2 or greater).
16. History of ascites, varices, variceal hemorrhage, or hepatic encephalopathy.
17. Any of the following pre-existing diagnoses, which are indicative of significant underlying liver disease, are present in the medical record: portal hypertension, splenomegaly.
    a. A subject is not eligible if the serum albumin level is below the central laboratory's lower limit of normal;

       AND

    b. FibroTest/FibroSURE with a result > 0.48. Subjects with borderline Fibrosure assessments may be enrolled after confirmation by the Sponsor's Medical Monitor. Of note, if a subject has a known history of Gilbert's syndrome, A FibroTest cannot be used for fibrosis testing.

18. Markers of hepatic inflammation or cirrhosis as evidenced by 1 or more of the following:
    a. Platelet count < 150,000/μL.
    b. Total bilirubin > 1.5x ULN and direct bilirubin ≥ 0.5 mg/dL.
    c. ALT or AST > 1.0x ULN.
    d. Alkaline phosphatase > 2.0x ULN.
19. Prothrombin time INR ≥ 1.4.
20. Serum creatinine > 1.5 mg/dL.
21. HIV if $CD4^+$ cell count ≤200 mm^3 and/or viral load >20 copies/mL.
22. Urine protein > 30 mg/dL.
23. Body mass index > 38.
24. Major surgery planned within 6 months after enrollment.
25. Acute or chronic disease that, in the opinion of the Investigator, would adversely affect subject safety or compliance or interpretation of study results.
26. Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0.
27. Significant cardiovascular disease (such as New York Heart Association Class III or IV cardiac disease, congestive heart failure, myocardial infarction within the previous 6 months, unstable arrhythmias, or unstable angina) or significant pulmonary disease (including obstructive pulmonary disease).
28. History of arterial or venous thrombosis / thromboembolism, or a known pro-thrombotic condition.
29. Recent history of psychiatric illness or cognitive dysfunction (including drug or alcohol abuse) that, in the opinion of the Investigator, is likely to impair subject's ability to comply with protocol mandated procedures.
30. Concurrent enrollment in another clinical study, unless it is an observational (non-interventional) clinical study that does not interfere with the requirements of the current protocol or have the potential to impact the evaluation of safety and efficacy of SHP648 and with prior consultation with the Sponsor's Medical Monitor
31. Subject is family member or employee of the Investigator.

5.3 Restrictions

Not applicable for this study.

5.4 Reproductive Potential

5.4.1 Male Contraception

The germline transmission of AAV8 and AAV2 was comparatively investigated in rabbits by Favaro et al., 2009. The results revealed a transient presence of AAV in the semen, which was vector dose-dependent but serotype-independent. Upon fractionation, the AAV was not present in motile sperm, greatly diminishing the risk of vertical transmission of vector DNA sequences. Furthermore, long-term follow-up showed that there was no recurrence of vector sequences in semen. The risk of transmission was also limited since infectious vector particles were detectable only up to Day 4 post injection. Moreover, since the ejaculate contains only 5% sperm and the AAV shedding into semen was comparable in vasectomized and non-vasectomized animals, the authors stated that the presence of AAV in semen does not necessarily imply transduction of germ cells and the associated risk of germline transmission (Favaro et al., 2009).

Based on the experience of Favaro et al., and others (Arruda et al., 2001, Couto et al., 2004, Favaro et al., 2009, Schuettrumpf et al., 2006) and the observed minimal distribution of SHP648 to testes in mice during the nonclinical development program for SHP648, sexually active men participating in Study SHP648-101 must agree to use a condom during sexual intercourse or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners in the period from SHP648 administration until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least 2 consecutively collected semen samples assessed at the central laboratory (this is applicable also for subjects who are surgically sterilized).

Refer to Appendix 4 for further guidance.

6. STUDY INTERVENTION

6.1 Investigational Product

6.1.1 Identity of Investigational Product

The IP is SHP648, which contains a single-stranded adeno-associated virus (ssAAV) genome consisting of liver-specific cis-regulatory modules (CRM8), a modified (truncated) murine transthyretin (pre-albumin) enhancer / promoter region, an intron derived from the minute virus of mice (MVM), a human codon-optimized coagulation factor 9 (*F9*) Padua transgene and a bovine growth hormone polyadenylation sequence (BGH polyA). The insert is flanked by adeno-associated virus serotype 2 (AAV2) inverted terminal repeat (ITR) sequences. The AAV serotype 8 (AAV8) capsid harboring the vector genome is composed of the viral structural proteins VP1, VP2, and VP3.

SHP648 will be supplied as a 5 mL clear and sterile frozen aqueous solution in glass vials intended for IV infusion. The final product formulation consists of a solution of SHP648 in 10 millimolar (mM) histidine, 100 mM NaCl, 50 mM glycine, 5% trehalose and 0.005% polysorbate (Tween®) 80. Additional information is provided in the most current SHP648 Investigator's Brochure.

The product is filled at a target (nominal) concentration of about 4.1 x 10^{12} vg/mL as described on the vial label and will be provided to the sites in 10R vials, which contains 5 mL of the IP. The dosing shall be calculated based on the actual concentration (to be retrieved from the certificate of analysis) and based on the subject's body weight (BW).

6.1.2 Blinding the Treatment Assignment

Not Applicable as it is an open label study

6.2 Administration of Investigational Product

6.2.1 Dosing

Each patient will be assigned to one cohort only and will receive the respective assigned dose. This dose is to be administered once per patient as a peripheral intravenous infusion. For a detailed step by step guidance on handling and administration of the study drug, refer to the instructions in the SHP648 Pharmacy Manual.

Briefly, a qualified pharmacist and/or designee will thaw SHP648, pool the calculated amount into appropriate syringes, and provide the syringes to the investigator. The individual infusion volume will be based on the subject's assigned dose, the actual concentration listed on the certificate of analysis for the used lot and BW determined at the Screening Visit.

Initially, approximately 10% of the SHP648 infusion volume will be administered by manual push followed by an observation period of 30 minutes to monitor for infusion reactions. The remaining product will then be administered as specified in the SHP648 Pharmacy Manual.

6.2.1.1 Dose Escalation Plan

Up to 3 SHP648 dose cohorts are planned to be evaluated in this study, where the dosing from one cohort to the next is increased based upon the results of the previous cohort.

- Cohort 1 dose:
  - Starting dose: 4.0×10^{11} vg/kg BW
- Cohort 2 dose:
  - 2-fold escalation: 8.0×10^{11} vg/kg BW

    OR
  - 3-fold escalation: 1.2×10^{12} vg/kg BW
- Cohort 3 dose:
  - 2-fold escalation of Cohort 2 dose
  - 1.6×10^{12} vg/kg BW (if cohort 2 dose was 8.0×10^{11} vg/kg BW) or
    2.4×10^{12} vg/kg BW (if cohort 2 dose was 1.2×10^{12} vg/kg BW)

    OR
  - 3-fold escalation of Cohort 2 dose
  - 2.4×10^{12} vg/kg BW (if cohort 2 dose was 8.0×10^{11} vg/kg BW) or
    3.6×10^{12} vg/kg BW (if cohort 2 dose was 1.2×10^{12} vg/kg BW)

Initially, one subject will be dosed in Cohort 1, followed by an observational period of at least 6 weeks. If no safety concern is observed in the first dosed subject, a second subject will be dosed. For each additional dose cohort, dosing of the second subject will be scheduled 6 weeks after dosing of the first subject at the earliest; up to 7 subjects may be dosed in Cohort 1, if Cohort 1 is expanded.

Decisions pertaining to cohort expansion, dose escalation, number of subjects to be dosed, as well as the exact dose for subsequent Cohort 2 and Cohort 3 will be made based on the recommendation provided by an external Data Monitoring Committee (DMC). The DMC will review data including the Week 14 visit of the second dosed subject in Cohort 1.
The observation period for the DMC data cut in expanded Cohort 1 and for Cohorts 2 and 3 may be adjusted to between 6 and 14 weeks based on Cohort 1 FIX expression peak data observed in the first two patients, as well as, other relevant study data and published literature.

After review of all safety and FIX activity data, the DMC will provide recommendations to pursue one of the following:

1. Escalate to the next dosing cohort
2. Increase the number of subjects in the current dosing cohort
3. Cap enrollment at the current dosing cohort and complete the study
4. Enroll additional subjects into a previous lower dose cohort in order to obtain additional safety information
5. Enroll subjects into a modified dosing cohort that is intermediate between those into which subjects have been previously enrolled.

6.2.1.2 Stopping Criteria for Enrollment and Dose Escalation

Enrollment and escalation to next dose cohort will be paused until an independent DMC evaluates all available study data and makes a recommendation if any one of the following criteria are met:

1. Achievement of vector-derived FIX activity level ≥ 150% in any subject at any time while on the study due to potential risk of thrombosis. The level of FIX activity is measured frequently throughout the study. Study enrollment will be paused based on the local laboratory results (Table 1 and Table 2).
2. First two subjects on corticosteroid prophylaxis experience, at week 14, a loss of endogenous FIX activity level >75% of the highest FIX activity level measured after SHP648 administration (based on one stage central laboratory). The level of FIX activity is assessed frequently throughout the study (Table 1 and Table 2).
3. A value of 3x ULN or greater in ALT, AST, or both in any subject after SHP648 administration that is not responsive after 12 weeks of corticosteroid rescue treatment.
4. A SAE that may be potentially related to SHP648 and which poses either an immediate risk to the subject's health or is likely to adversely affect the subject's health long term. This includes events classified as AEs qualifying for special notification (Brussels, 03/12/2009, ENTR/F/2/SF/dn D(2009) 35810; EMEA/CHMP/GTWP/60436/2007), if these are judged as potentially related to SHP648.
5. The development in any subject of an inhibitor towards FIX (or FIX Padua) after having received SHP648. Further investigations of the characteristics and potential contributing factors and causal relationships of the observed FIX (or FIX Padua) inhibitor will be initiated.

6. Death of subject after having received SHP648, that is judged as, definitely, probably or possibly attributed to SHP648. Enrollment in the study and further dosing will be temporarily stopped in order to undergo review by the applicable regulatory authorities and the DMC.
7. Occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to SHP648.
8. Occurrence of moderate or severe-drug-related AEs in 2 or more SHP648 treated subjects in a given cohort.
9. If any other drug-related event occurs in SHP648 treated subjects and is deemed to pose an unacceptable risk to subjects by the investigator or medical monitor after further evaluation, additional subjects will not be enrolled or dosed until a decision is taken to stop or proceed with the study based on further evaluation of the available data by the DMC. Following a safety review of the event, study enrollment or dosing of subjects in the screening period may be restarted if the medical monitor and the investigator determine that it is safe to proceed with the study.

Study enrollment or dosing of currently enrolled subjects may be restarted if the DMC considers that it is safe to proceed with the study. All the applicable rules regarding DMC decisions are addressed in the DMC Charter.

6.2.1.3 Data Monitoring Committee (DMC)

The DMC is an external independent body that will consist of recognized medical experts in the fields of Hematology, Hepatology, Clinical Immunology and Gene Therapy. The DMC will act as an advisory body to the Sponsor that will monitor subject safety and recommend whether the study should continue, be modified or stopped. Responsibilities of the DMC members and all the applicable rules regarding DMC decisions are addressed in the DMC Charter.

6.2.2 Dose Modification

The decision to proceed to the next dose level will be triggered after DMC review of safety, tolerability, and preliminary FIX expression data obtained in at least 2 participants at the prior dose level.

The DMC will monitor the safety during the study, and will have standing to approve or stop dose escalation, expand a dose cohort to obtain more safety data, cap enrollment at the current dosing cohort and complete the study, enroll additional subjects into a previous lower dose cohort in order to obtain additional safety information, modify or add a dose escalation in between planned dose cohort, and add a dose to a treatment regimen, modify observation period for the DMC data cut. (refer also to Section 6.2.1.1 and Section 6.2.1.2).

6.3 Labeling, Packaging, Storage, and Handling of Investigational Product

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

The IP is labeled with a minimum of the following: protocol number, product identification number, dosage form, directions for use, storage conditions, expiry date, lot number, the statements "For clinical trial use only" and "Contains genetically modified organisms" and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Space is allocated on the label so that the site representative can record the Subject ID number. Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The IP is filled in glass vials in volumes of 5mL with an approximate concentration of nominal 4.1×10^{12} vg/mL. Each single vial is packed in a carton, which is labelled as well.

Changes to sponsor-supplied packaging prior to dosing may not occur without full agreement in advance by the sponsor.

6.3.3 Storage

SHP648 will be stored at -60°C or below. The investigator has overall responsibility for ensuring that investigational product is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented. The pharmacist/nominated team member will enter the unique subject identifier on the investigational product bottle label and the outer carton label as they are distributed.

The IP must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the investigational product is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (i.e., certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion from the established range. Temperature excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the investigational product and will provide supportive documentation as necessary.

Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP, e.g., fumigation of a storage room.

6.3.4 Special Handling

Handling and disposal of SHP648 will comply with standards for Biosafety Level 1 Vectors as well as universal precautions.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the investigational product to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the investigational product, documenting shipment content and condition. Accurate records of all investigational product dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing investigational product. Where permissible, tasks may be delegated to a qualified designee (e.g., a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will administer/dispense the investigational product only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the investigational product carrying his/her treatment assignment. All administered/dispensed medication will be documented in the subject's source and/or other investigational product record. No investigational product stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records.

At the end of the study, or as instructed by the sponsor, all unused stock, are to be sent to a nominated contractor on behalf of the sponsor. Investigational products being returned to the sponsor's designated contractors must be counted and verified by clinical site personnel and the sponsor (or designated CROW). For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled

amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Returned investigational products must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any investigational product prior to shipment. Shipment of all returned investigational product must comply with local, state, and national laws.

Based on entries in the site drug accountability forms, it must be possible to reconcile investigational products delivered with those used and returned. All investigational products must be accounted for and all discrepancies investigated and documented to the sponsor's satisfaction.

6.5 Subject Compliance

All study procedures are to be performed under the direct supervision of the investigator/a licensed healthcare professional at the study site, and thus, no separate procedures will be used to monitor subject compliance.

6.6 Prior and Concomitant Therapy

All medications taken and non-drug therapies other than study treatment (including but not limited to FIX concentrates, herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 4 weeks prior to the Screening Visit (or pharmacokinetic equivalent of 5 half-lives, whichever is longer) and through the final study contact (including protocol-defined follow-up period) and the dose or amount taken must be recorded in the subject's source document and on the concomitant medications and non-drug therapies electronic case report forms (eCRFs).

6.6.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to FIX concentrates, herbal treatments, vitamins, non-pharmacological treatment such as psychotherapy as appropriate) received within 4 weeks prior to the Screening Visit. Prior treatment information must be recorded in the subject's source document.

6.6.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of investigational product and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded in the subject's source document.

6.6.3 Permitted Treatment

The following medications and non-drug therapies are permitted **within 4 weeks** before study entry and during the course of the study (refer also to Section 6.6.4, Table 5):

- Medications:
  - Antiretroviral therapy.
  - Antihistamines, nonsteroidal anti-inflammatory drugs, acetaminophen, and corticosteroids by oral or parenteral route for the treatment of allergic reaction.
  - Hemostatic agents, such as tranexamic acid, are permitted, as indicated by the subject's treating physician, to treat mucosal bleeding during the study.
  - FIX products (refer to Section 6.6.3.1).
  - Any medications deemed necessary by the subject's physician to treat or prevent any medical condition (with the exception of any immunomodulating drug* other than anti-retroviral chemotherapy, and any investigational drug or device).
  - Any over-the-counter medication used by the subject to treat symptoms or signs of any medical condition.
  - Supplemental vitamins, minerals.
- Non-drug therapies:
  - Any non-drug therapy (e.g., physiotherapy) deemed necessary by the subject's physician to treat or prevent any medical condition.

* In general, it is the intention of the protocol to avoid the use immunomodulating drugs during the first 12 months following the SHP648 infusion as these may interfere with the evaluation of the immune response to SHP648. A clear exception is the use of corticosteroids, which has become recognized as a treatment element in gene therapy for patients exhibiting elevated liver enzymes. The risk-benefit of vaccination needs to be evaluated on the individual basis as the expected immune response to the vaccine may also confound the evaluation of the immune response to SHP648.

6.6.3.1 Use of Exogenous FIX throughout the Study

Clinical bleeding episodes will be managed with infusion of exogenous FIX. Usage of exogenous FIX for the prevention (prophylactic treatment in case of FIX activity levels <2% and other cases when necessary at the discretion of investigator and medical monitor) or episodic management (on-demand FIX treatment) of hemorrhages will be at the discretion of the investigator and/or local hemophilia physician, and in consultation with the study subject. However, in order to assess FIX activity levels derived specifically from the SHP648 transgene and not exogenous FIX products, subjects will be asked to refrain from prophylactic FIX usage between Week 3 to Week 14 post-SHP648 administration and during the 3 weeks prior to Month 9.

During these periods which are critical for the evaluation of sustained FIX expression from the vector (as both an efficacy and safety evaluation), on-demand treatment that is required for clinical bleeding should, if possible, be performed using a standard half-life FIX product (rather than an extended half-life FIX concentrate). Whenever possible, a 5- day wash out period should be observed prior to any study visit with a FIX assessment, however, scheduled visits should not be missed in the case of a wash out of less than 5 days.

Prophylactic regimen with exogenous FIX may be resumed after Week 14 in case of FIX activity levels <2% and/or at the discretion of the Investigator in consultation with the Sponsor's Medical Monitor, for subjects in whom gene therapy is insufficient to control or prevent spontaneous bleeds.

It is recognized and expected that suspected or evident bleeding episodes will be treated with infused FIX protein as per the standard of care. If possible, study visits should be scheduled at least 5 days after a FIX dose to minimize the chance that any FIX activity measured at the study visit results from exogenously administered FIX. However, with the exception of the Screening Visit, study visits should not be postponed if the study subject has infused FIX (e.g., for the treatment of bleeding) within 4 days of the scheduled study visit.

All use of exogenous FIX will be recorded in the source document and eCRF.

6.6.3.2 Glucocorticoid Use in the Study

Should two subjects in any dosing cohort, experience hepatic inflammation requiring reactive glucocorticosteroid treatment, subjects who are subsequently dosed with SHP648 will receive prophylactic prednisolone (or prednisone) treatment starting at Day 8 after SHP648 infusion. Prophylactic treatment should follow the regimen proposed below in Table 4. The corticosteroid regimen may be adjusted at the discretion of the Investigator in consultation with the Sponsor's Medical Monitor depending on the subject's tolerance of the regimen and the observed hepatic transaminase response. Patients receiving corticosteroids may require additional visits to evaluate potential side effects of corticosteroid therapy and for dose adjustments, FVIII activity and liver function tests (LFTs) will be measured at these visits by local laboratories. If a subject does not tolerate corticosteroid therapy, per the AASLD guidelines combination therapy with azathioprine/prednisone should be used. This combination therapy allows lower cumulative glucocorticoid dose and risk.

Notwithstanding the protocol language requiring that two subjects must first be eligible for reactive corticosteroid treatment, the Sponsor Clinical Director with the concurrence of the investigator, may permit the use of prophylactic steroids in any subject based on all available scientific evidence at the time of subject enrollment.

Such evidence may include the clinical outcomes of others AAV-based gene therapy studies as well as nonclinical relevant data that may emerge. The possible use of corticosteroids will be communicated to potential subjects as part of the ICF process.

Glucocorticoid Treatment

A tapering course of glucocorticosteroid treatment initiated with 60 mg/day of prednisolone will be employed:

- **Reactively**, if any of the following criteria are met:
  a. A subject demonstrates an apparent vector-associated FIX expression of greater than 3-fold baseline activity level (e.g., > 3-6% FIX activity level) followed by a loss of > 66% of any confirmed FIX activity value over a 1- to 2-week period,

     AND/OR

  b. A subject demonstrates a clinically significant ALT elevations defined as:
     - 1.5 x baseline value at Screening Visit

       OR

     - above the upper limit of normal (ULN).
- **Prophylactically** at Day 8 after SHP648 infusion, if two subjects from the same dosing cohort experience hepatic inflammation requiring reactive glucocorticosteroid treatment.

In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (e.g. USA), prednisone will be provided. Prednisone is converted to the active metabolite prednisolone and is recommended by the AASLD and the EASL for the treatment of autoimmune hepatitis. Of note, in addition to central laboratory FIX activity levels, local laboratory FIX activity values may be used to inform the initiation of corticosteroid treatment (European Association for the Study of the Liver, 2015).

A tapering course of corticosteroids (prednisolone or prednisone depending on which product is approved in the region) will be given to interrupt any evidence of clinical or subclinical hepatic inflammation and to support the potential for sustained transgenic FIX expression. It is imperative to begin dosing with prednisolone as soon as possible after either of the prednisolone-triggering observations listed above has been made, preferably within 12-24 hours of the recognition of transaminitis or decreasing FIX activity. If a confirmatory blood sample can be collected, this information will be useful in the management; however, the initiation of prednisolone therapy should not be delayed pending the opportunity for sample collection.

The prednisolone dosing regimen described in Table 4 is provided as a guidance for the dosing regimen with corticosteroids and must be tailored to the individual subject depending on the tolerance of the regimen and the observed hepatic transaminase response at the discretion of the Investigator and in consultation with the Sponsor's Medical Monitor.

Table 4. Guidance for Prednisolone Dosing Regimen

Prednisolone Dose (mg/day)	Duration
60	1 week or more until AST/ALT decline is observed
40	1 week
30	1 week
25	1 week
20	1 week
15	1 week
12.5	2 weeks
10	2 weeks
5	1 week

Source: Terziroli Beretta-Piccoli et al., 2017

Note: Determine ALT and FIX activity weekly or twice weekly as per study protocol.

Careful monitoring for known side effects of prolonged exposure to Prednisolone should be maintained, including:

- low bone mineral density (osteoporosis),
- osteonecrosis,
- adrenal suppression,
- hyperglycemia and diabetes;
- sleep and psychiatric disturbances.

If a subject does not tolerate corticosteroid therapy consideration should be given to combination therapy with azathioprine/corticosteroid per AASLD guidelines or other drug which allows lower cumulative glucocorticoid dose and risk as per standard of practice, in consultation with a hepatologist, so that the care can be individualized to the subject's needs.

6.6.4 Prohibited Treatment

*The following medications and non-drug therapies are **not** permitted within 30 days before the SHP648 dose (unless stated otherwise):*

- Systemic antiviral and/or interferon therapy (with the exception of antiretroviral therapy).
- Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders.
- An investigational intervention within 4 weeks prior to dosing or within 5 half-lives of the investigational drug administration, whichever is longer.

  Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0
- Non-drug therapies:
  - Major surgery or an orthopedic surgical procedure planned within 6 months after dosing.

A subject who has taken any of these medications or anticipates receiving any of these non-drug therapies within the specified timeframe will be recorded as a protocol deviation.

The wash out periods of common prior treatments that are excluded medications in this study are presented in Table 5.

Table 5. Common Excluded Treatments and Associated Washout Period

	Minimum Number of Days BEFORE First Dose		Minimum Number of Days AFTER First Dose
Treatment	**30**	**365**	**180**
Systemic antiviral therapy [a]	X		
Interferon therapy[a]	X		
Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders	X		
AAV vector or any other gene transfer agent		X	
Investigational intervention [b]	X		
Planned major surgery or an orthopedic surgical procedure			X

[a.] with the exception of antiretroviral therapy
[b.] or within 5 half-lives of the investigational drug administration, whichever is longer.

Treatments not listed above are considered allowable.

7. DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Participation

If study participation is discontinued (i.e., complete withdrawal from study participation), regardless of the reason, the evaluations listed for the Early Termination Visit should be performed as completely as possible. Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for discontinuation and date of discontinuation from study participation must be recorded in the source documents and the End of Study eCRF.

Subjects who received SHP648 and who then discontinue/withdraw will not be replaced.

7.2 Reasons for Discontinuation

The reason for discontinuation must be determined by the investigator and recorded in the subject's source document and on the End of Study eCRF. If a subject is discontinued for more than 1 reason, each reason should be documented in the source and the most clinically relevant reason should be indicated in the eCRF.

Reasons for discontinuation include, but are not limited to:

- Adverse event (experienced prior to dosing that prevents further participation)
- Withdrawal by subject
- Lost to follow-up
- Other - If "Other" is selected, the investigator must specify on the eCRF.

7.3 Withdrawal from the Study

Any subject may voluntarily withdraw (i.e., reduce the degree of participation in the study) consent for continued participation and data collection. A subject may withdraw from the study at any time and for any reason without prejudice to his/her future medical care by the physician or at the institution. The reason for withdrawal will be recorded on the End of Study eCRF. Assessments to be performed at the termination visit.

Subjects will also be withdrawn prior to SHP648 dosing for the following reasons:

- The subject develops a confirmed FIX inhibitory antibody (≥0.6 Bethesda units (BU) by Nijmegen modification of the Bethesda assay).
- The subject is non-compliant with study procedures, in the opinion of the investigator.

7.4 Subjects "Lost to Follow-up" Prior to the Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject who is lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that the subject return to the site for final safety evaluations.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Study Periods

Refer to Table 1 for the schedule of study activities. Study assessments are detailed in Section 8.2 and Table 2.

Any subject who provides informed consent (i.e., signs and dates the informed consent form [ICF]) is considered a subject in the study.

The written informed consent for all procedures and assessments for the conduct of the study must be obtained prior to any study related procedure. If the required 5-day wash out period falls into the routine praxis, the ICF can be signed at the Screening Visit, otherwise it has to be signed before wash-out.

Every single subject, who enters the study will get a unique subject identification code (SIC). Subjects who fail to meet eligibility criteria may be re-screened; they will be assigned a different SIC at re-screening.

8.1.1 Screening Visit

Prior to the Screening Visit, all subjects will undergo a minimum wash out period of 5 days following their last FIX therapy (on-demand or prophylaxis), after which blood samples will be collected to determine eligibility. Subjects will undergo the screening visit (up to 70 days before Day 0) prior to dosing with SHP648 (Day 0). An enrollment period of 45 days is planned for each cohort.

FIX activity levels at the Screening Visit determined by the central laboratory with the one-stage clotting assay will be used as baseline FIX activity levels.

The following procedures and assessments will be performed:

- Availability of signed informed consent
- Eligibility criteria checked
- Demographic information
- Full Physical exam (examination of general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological), including height and weight
- Medical history including hemophilia history (confirmation of diagnosis and severity; presence of any target joints; historical annualized bleed rate (ABR) based on documented data within the last 12 months), and documentation of all FIX replacement therapies and other medications used within the last 12 months.

Medical history to also include any surgeries, hospitalizations within the last 12 months, exposure to mutagenic agents, recent malignancy(ies), recent incidence or exacerbation of a pre-existing neurologic disorder, recent incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, recent incidence of hematologic disorder.

- 12-lead ECG
- Vital signs
- Blood, bodily fluids and stools for laboratory assessments (Table 2):
  - Hematology, coagulation and clinical chemistry panels
  - Activated partial thromboplastin time (aPTT)
  - Local Laboratory Liver Function Tests (LFTs) including ALT, AST, and GGT
  - Hepatitis B surface antigen, hepatitis C antibody, hepatitis C virus (HCV) RNA, markers of autoimmune-mediated hepatitis
  - FIX genotyping, FIX activity level (central and local laboratories), FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product
  - Neutralizing and binding antibodies to AAV8 and AAV2
  - Cell-mediated immune (CMI) response to AAV8 and FIX transgene products
  - [redacted]
  - FibroSURE™ and urine protein
  - Saliva, urine, stool, and semen collected for PCR of vector genomes
  - Human leucocyte antigen (HLA) (MHC haplotype), human immunodeficiency virus (HIV) serology
  - Blood collected for backup
  - Blood collection for a possible whole exome sequencing if subject provides consent
- Distribute patient diaries (paper diary) to capture subject reported data including bleeding episodes and FIX usage
- [redacted]
- [redacted]
- [redacted]

A **screen failure** is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered investigational product(s). The study site is responsible for maintaining an enrollment/screening log that includes all subjects enrolled. The log also will serve to document the reason for screening failure. If a subject does not satisfy all screening criteria, the same subject may be re-screened at a later date. A complete or partial re-screen may also become necessary at the discretion of the investigator or sponsor.

All screening data will be collected and reported in eCRFs, regardless of screening outcome. For the purpose of analysis, only the data from the most recent screening outcome will be used. If a subject is re-screened after a prior screening failure, the End of Study eCRF should be completed, and a new ICF, new SIC and new eCRF are required for that subject. Exemptions may be granted for administrative reasons (e.g. delay in timely availability of laboratory results).

8.1.2 Treatment Period

8.1.2.1 Day 0

On Day 0 visit, eligibility criteria will be reviewed.
For subjects who meet all inclusion and exclusion criteria the following procedures and assessments will be performed:

- Targeted physical exam (includes weight and examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs (up to 3 hours pre-infusion and at 5, 15, and 30 mins and 1, 2, 3, 4, 6, and 8 hours post-infusion)
- SHP648 administration (single infusion)
- Blood collected for laboratory assessments
  - Hematology, coagulation and clinical chemistry panels
  - aPTT (pre-SHP648 infusion)
  - Serum cytokines (including IL-6 and TNF-α).
  - FIX activity (central and local laboratories) and FIX antigen (pre-infusion [up to 3 hours prior to infusion])
  - Neutralizing and binding antibodies to AAV8 and AAV2 (up to 3 hours pre-infusion)
  - Blood collected for backup (up to 3 hours pre-infusion and 8 hours post-infusion)
  - If patient provided consent, sample collection for a possible transcriptome testing (up to 3 hours pre-infusion and 8 hours post-infusion)

  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available

- Collect/ review patient diaries
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs (i.e., Untoward Medical Occurrences at Day 0 Visit)
- Changes in intensity of physical activity

8.1.3 Follow-Up Period

The follow-up period for this protocol is 12 months after the dosing visit (Day 0 Visit). At the end of this period, there will be a study completion visit (refer to Section 8.1.3.6, Table 1 and Table 2).

All AEs and SAEs that are not resolved at the time of Study Completion will be followed to closure (see Section Appendix 3.2).

8.1.3.1 Day 1

The following procedures and assessments will be performed:

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - Serum cytokines (including IL-6 and TNF-α)
  - FIX activity (central and local laboratories)
  - Saliva, urine, stool, and semen collected for PCR of vector genomes
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- 12-lead ECG

- Collect/ review patient diaries
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity

8.1.3.2 Week 1 to Week 3

The following procedures and assessments will be performed at the first visit of the week (**Clinic Visit**):

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - HCV RNA (Week 1 only)
  - FIX activity (central and local laboratories) and FIX antigen
  - FIX inhibitor
  - neutralizing and binding antibodies to AAV8 and AAV2 (Week 2 only)
  - CMI response to AAV8 and FIX transgene products
  - [redacted]
  - Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points)
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic

- AEs
- Changes in intensity of physical activity

8.1.3.3 Week 4 to Week 14

The following procedures and assessments will be performed at the **Clinic Visit** (first visit of the week):

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments:
  - Hematology (Weeks 5, 7, 9, 11, & 13)
  - Coagulation (Weeks 5, 7, 9, 11, & 13)
  - Clinical chemistry panels (Weeks 5, 7, 9, 11, & 13)
  - aPTT
  - LFTs
    - Central Laboratory LFTs @ Clinic Visits during Weeks 4, 6, 8, 10, 12, and 14
    - Local Laboratory LFTs @ every Clinic Visits during Weeks 4-14.
  - HCV RNA (Week 6 only)
  - FIX activity (central and local laboratories), FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product (Weeks 6 and 8 only)
  - Neutralizing and binding antibodies to AAV8 and AAV2 (Weeks 4, 8, and 12 only)
  - CMI response to AAV8 and FIX transgene products
  - [redacted]
  - Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points)
- Collect/ review patient diaries (Week 4, then every 2 weeks)
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity

The following assessments will be performed weekly at every **Lab Visit** (second visit of the week):

- Blood collected for laboratory assessments:
  - aPTT
  - LFTs
    - Local Laboratory LFTs
  - FIX activity (central and local laboratories).
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available.

8.1.3.4 Week 15

The following procedures and assessments will be performed (**Clinic Visit** only):

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - FIX activity (central and local laboratories), FIX antigen
  - FIX inhibitor
  - CMI response to AAV8 and FIX transgene products
  - Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points)
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries
- Concomitant medications (including FIX usage)

- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity

8.1.3.5 Month 4 to Month 6 and Month 9

The following procedures and assessments will be performed monthly:

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - FIX activity (central and local laboratories) and FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product (Months 6 and Month 9 only)
  - Neutralizing and binding antibodies to AAV8 and AAV2
  - CMI response to AAV8 and FIX transgene products (Months 4, 5, and Month 6 only)
  - Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points)
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries (to be reviewed every 2 weeks)
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity
- [redacted]
- [redacted]

8.1.3.6 Study Completion Visit (Month 12)

The following procedures and assessments will be performed at the Study Completion Visit:

- Full Physical exam (examination of general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological), including height and weight
- Vital signs
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - HCV RNA
  - FIX activity (central and local laboratories) and FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product
  - Neutralizing and binding antibodies to AAV8 and AAV2
  - CMI response to AAV8 and FIX transgene products
  - [redacted]
  - FibroSURE (Month 12 only)
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity
- [redacted]
- [redacted]
- [redacted]

For tabular summaries of activities and assessment to be performed at the Study Completion Visit refer to Table 1 and Table 2.

8.1.3.7 Unscheduled Visit

Unscheduled visits will be performed if a >50% decrease in FIX activity from that of the last visit is observed at any time after Week 14. FIX activity levels and liver enzyme levels will be assessed at these visits.

The following procedures and assessments will be performed:

- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints)
- Vital signs
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels,
  - aPTT
  - HCV RNA
  - FIX activity (central and local laboratories) and FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product
  - Neutralizing and binding antibodies to AAV8 and AAV2
  - CMI response to AAV8 and FIX transgene products
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome analysis
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries.
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity

8.1.3.8 Early Study Termination Visit

The following procedures and assessments will be performed at the Early Termination Visit (including in cases of withdrawal or discontinuation):

- Full Physical exam (examination of general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological), including height and weight
- Vital signs
- Blood, bodily fluids and stools collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels
  - aPTT
  - HCV RNA
  - FIX activity (central and local laboratories) and FIX antigen
  - FIX inhibitor
  - Antibodies to FIX and FIX transgene product
  - Neutralizing and binding antibodies to AAV8 and AAV2
  - CMI response to AAV8 and FIX transgene products
  - [redacted]
  - FibroSURE
  - Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points)
  - Blood collected for backup
  - If patient provided consent, sample collection for a possible transcriptome testing
  - If patient provided consent, a possible metabolomics analysis can be done provided plasma / serum backup sample is available
- Collect/ review patient diaries
- Concomitant medications (including FIX usage)
- Bleeding episodes, spontaneous and traumatic
- AEs
- Changes in intensity of physical activity
- [redacted]

- [redacted]
- [redacted]

For tabular summaries of activities and assessment to be performed in case of early termination refer to Table 1 and Table 2.

8.1.4 Additional Care of Subjects after the Study

No aftercare is planned for this study. Subjects completing the current Phase 1/2 Study (SHP648-101) will be highly encouraged to enroll seamlessly into a follow-up interventional study (Extension Study), in which they will be observed for an additional 4 years.

8.2 Study Assessments

8.2.1 Demographic and Other Baseline Characteristics

Subject demographic information including gender, age, and race will be collected prior to the subject being administered the SHP648 infusion.

8.2.1.1 Height and Weight

Height (in or cm) and weight (lb or kg) will be measured and recorded in the physician's source documents.

8.2.1.2 Medical and Medication History

Medical and medication history will be collected and recorded in the physician's source documents. At the Screening Visit, the subject's medical history will be described for the following body systems including severity (defined in Appendix 3) or surgery and start and end dates, if known: eyes, ears, nose, and throat; respiratory; cardiovascular; gastrointestinal; musculoskeletal; neurological; endocrine; hematopoietic/lymphatic; dermatological; and genitourinary. History will also include hemophilia history (confirmation of diagnosis and severity; presence of any target joints; historical ABR based on documented data within the previous 12 months) and documentation of all FIX replacement therapies and other medications used within the last 12 months.

The historical ABR including treated and untreated bleeding episodes in the 12 months prior to study enrollment based on medical records should also be recorded in the eCRF.

Hospitalizations, exposure to mutagenic agents, recent malignancy(ies), recent incidence or exacerbation of a pre-existing neurologic disorder, recent incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and recent incidence of a hematologic disorder will be recorded from 4 months before providing informed consent up to study completion/discontinuation (refer to Table 1).

All medications taken and non-drug therapies received, from 4 weeks before providing informed consent until completion/termination will be recorded on the concomitant medications and non-drug therapies eCRFs.

8.2.2 Efficacy

8.2.2.1 Circulating Plasma FIX Activity and FIX Antigen Levels

The following assessments will be made at the time points presented in Table 1 and Table 2 as part of the analysis and interpretation of efficacy outcome measures:

- FIX activity (Central Laboratory: one-stage clotting AND chromogenic assays; Local Laboratory: one-stage clotting OR chromogenic assay).
- FIX antigen.

8.2.2.2 Number of Bleeding Episodes Post-SHP648 Infusion

The number of bleeding episodes post-SHP648 administration will be assessed based upon each individual bleeding episode, spontaneous or traumatic, recorded in the subject's paper diary, and/or recorded in the physician/nurse/study site notes. Documented bleeding episodes in the 12 months prior to study enrollment will also be recorded in the eCRF

A bleed is defined as subjective (e.g., pain consistent with a joint bleed [see Appendix 6]) or objective evidence of bleeding which may or may not require treatment with FIX. Bleeding episodes occurring at the same anatomical location (e.g., right knee) with the same etiology (e.g., spontaneous vs. injury) within 72 hours of onset of the first episode will be considered a single bleeding episode. If a bleed occurs following resolution of the bleed, it will be considered to be a "new" bleed and recorded accordingly. Bleeding occurring at multiple locations related to the same injury (e.g., knee and ankle bleeds following a fall) will be counted as a single bleeding episode.

Adverse events (AEs) and the details of concomitant medication use coincident with the treatment of all acute bleeding episodes will be recorded. Note that bleeding episodes are not to be reported as AEs (Appendix 3).

Hemophilia-related events meeting the criteria for seriousness will be reported as SAEs and described on the serious adverse event report (SAER) form.

8.2.2.3 Consumption of Exogenous FIX Compared to before Gene Transfer

Data pertaining to the consumption of exogenous FIX during the 12-month period prior to SHP648 infusion will be collected and recorded as part of the medical history. Post-gene transfer consumption of exogenous FIX will be measured at the time points indicated in Table 1.

Weight-adjusted consumption of exogenous FIX will be determined based upon the amount of exogenous FIX infused, as recorded in the subject's paper diary and the subject's weight, as measured at the study site.

8.2.3 Safety

8.2.3.1 Physical Examination

Physical examinations will be performed by the investigator. At the Screening Visit, the physical examination will be performed on all major organ systems (i.e., general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological and others as indicated). If an abnormal condition is detected at screening, the condition will be described on the medical history eCRF.

At subsequent study visits (as described in Table 1), a targeted physical examination will be performed (i.e., examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints). At study visits, if a new abnormal or worsened abnormal pre-existing condition is detected, the condition will be described on the AE eCRF. If the abnormal value was not deemed an AE because it was due to an error, due to a preexisting disease (described in Appendix 3), not clinically significant, a symptom of a new/worsened condition already recorded as an AE, or due to another issue that will be specified, the investigator will record the justification on the source record.

Abnormalities identified at the Screening Visit and at subsequent study visits will be recorded in the physician's source documents.

Patient Activity Level: In the event of a bleed, subjects will be asked to estimate their activity levels. Subjects will be asked whether there have been changes in the intensity of their physical activity.

8.2.3.2 Adverse Events

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (e.g., "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. Refer to Appendix 3 for AE definitions, assessment, collection time frame, and reporting procedures.

8.2.3.3 Vital Signs

Vital signs will include body temperature (°C or °F), respiratory rate (breaths/min), pulse rate (beats/min), and systolic and diastolic blood pressure (mmHg).

Vital signs will be measured at the Screening Visit, on Day 0 up to 3 hours before administration of IP and 5, 15, 30 minutes, 1, 2, 3, 4, 6, and 8 hours after administration of IP, at each subsequent study visit, and at study completion/discontinuation. Blood pressure will be measured when subjects are in the supine position.

Vital sign values are to be recorded on the eCRF. Additional tests and other evaluations required to establish the significance or etiology of an abnormal result or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the investigator.

The investigator will assess whether a change from baseline (as determined at the Screening Visit) in vital signs may be deemed clinically significant and whether the change should be considered and recorded as an AE.

8.2.3.4 Clinical Laboratory Tests

All clinical laboratory assessments (refer to Table 2) will be performed at a central laboratory, according to the laboratory manual. In addition, Liver Function Tests (ALT, AST, GGT) and FIX activity assessment will be performed by the local laboratory. The schedule for sample collection for laboratory analysis is described in Table 1 and Table 2.

All clinical laboratory tests will be performed according to the laboratory's standard procedures. Reference ranges will be supplied by the laboratory and used to assess the results for clinical significance and out-of-range changes which may be associated with, or constitute, an AE. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. The investigator's assessment of each laboratory value will be recorded on the eCRF.

Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

A complete list of the clinical laboratory tests to be performed is provided in the SHP648-101 Laboratory Manual.

8.2.3.4.1 Hematology, Coagulation, Clinical Chemistry, and Markers of Autoimmune Response

The hematology panel will consist of complete blood count (hemoglobin, hematocrit, erythrocytes [i.e., red blood cell {RBC} count], and leukocytes [i.e., white blood cell count]) with differential (i.e., basophils, eosinophils, lymphocytes, monocytes, neutrophils), platelet counts, RBC distribution width, mean corpuscular volume, and mean corpuscular hemoglobin concentration.

The coagulation panel will consist of PT, international normalized ratio, thrombin time, D-dimer, and fibrinogen activity. In addition, activated partial thromboplastin time, FIX antigen, FIX activity (performed as one-stage assay and as chromogenic assay) will also be assessed.

The clinical chemistry panel will consist of sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase (CK), bilirubin (total & direct), ALT, AST, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and lactate dehydrogenase (LDH).

The FibroSURE™ (Laboratory Corporation of America, Raritan, NJ, United States) will be measured at the Screening Visit and at Month 12 (Study Completion Visit). This score is based on an algorithm that includes following laboratory results: ALT, alpha-2-macroglobulin, apolipoprotein A1, total bilirubin, gamma-glutamyl transferase.

Markers of autoimmune-mediated hepatitis will include ANA, total IgG, anti-smooth muscle antibody, and anti-LKM1 titers. Serum cytokines will also be measured.

[redacted]

Blood will be obtained for assessment of hematology and clinical chemistry parameters and markers of autoimmune response at the study visits presented in Table 2. Hematology and clinical chemistry assessments will be performed on ethylenediamine-tetraacetic acid-anticoagulated whole blood and serum, respectively, at the central laboratory.
The FIX activity assay will be performed at both local and central laboratories. ALT (liver transaminases) will be performed at local laboratories at time points specified in Table 2 (i.e. Week 4 to Week 14).

8.2.3.4.2 Viral Serology

Viral serology testing will include HIV-1 and HIV-2 antibody, Hepatitis B core antibody, Hepatitis B surface antigen (HBsAg), Hepatitis C virus antibody and HCV RNA. The HCV titer will be confirmed by PCR for all subjects reported as HCV positive. All assessments will be performed at the Screening Visit. HCV RNA will also be performed at Week 1 and Week 6, and at the Early Study Termination Visit. Any positive test in HBsAg will be repeated using a new blood sample (refer to Table 2).

8.2.3.4.3 Genetic tests

The following tests are mandatory:

Assessment of *F9* gene mutations (FIX genotyping) and human leukocyte antigen (HLA) genotype (MHC haplotype) will be performed. of the following loci: HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRB1, and HLA-DRB3/4/5. Samples will be collected at the Screening Visit (refer to Table 2) and tested right away or at a later time. Analyses will be performed for the study at the central laboratory.

The results will be provided to the sites. The investigator will be responsible for informing the subject of the test results.

Further voluntary genetic tests are listed in Section 8.2.4.3.

8.2.3.4.4 Immunogenicity

The presence of binding antibodies to FIX, Padua FIX (FIX transgene product), inhibitor assays to FIX and Padua FIX, binding and neutralizing antibodies to AAV8 and AAV2, and CMI response to AAV8 and FIX transgene products based on an ELISPOT assay will be determined at the time points presented in Table 2.

Immunogenicity assessments for the proposed study will be carried out using validated assays performed by a qualified laboratory.

- Study subjects will be screened and monitored for the presence of AAV8 (vector used in this study) specific neutralizing antibodies and binding antibodies (IgG and IgM) at a central facility using a validated protocol. Subjects will also be tested for binding and neutralizing antibodies against AAV2 that infects humans with high prevalence.
- **CMI Assessment:** the AAV8 and FIX specific cell mediated immunity will be assessed using validated IFN-γ ELISPOT assays at screening and throughout the study period.

- **Binding antibodies to FIX or Padua FIX,** will be determined using validated FIX or **Padua FIX** specific enzyme-linked immunosorbent assay (ELISA). The assays will be performed at a qualified laboratory.
- **FIX Inhibitor Assay**: Development of antibodies that inhibit FIX activity (FIX Inhibitors) and Padua FIX will be measured at a central laboratory using a validated, clot-based, method following the Nijmegen modification of the Bethesda assay.

Immunogenicity values are to be recorded on the eCRF. For each value, the investigator will determine whether the value is considered an AE (see definition in Appendix 3 and record the sign, symptom, or medical diagnosis on the AE eCRF).

Additional tests and other evaluations required to establish the significance or etiology of an abnormal result, or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the investigator.

8.2.3.4.5 Vector Genome Shedding

Blood and bodily excretions will be obtained at the time points presented in Table 1 and assessed for presence of SHP648 genome until 2 consecutive measurements are negative. Quantitative PCR assays will be used to detect SHP648 vector genome in blood, saliva, urine, stool, and semen.

An infectious center assay is used as a quality control and part of the characterization of the stocks of the investigational product. No viral growth-based assay is used in the evaluation of the body fluids of the treated subject.

8.2.3.5 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) at rest will be performed at screening and at the Day 1 Visit and will be evaluated for medical significance by the investigator (refer to Table 1). 12-lead ECG values are to be recorded on the eCRF. For each value, the investigator will determine whether the value is considered an AE (see definition in Appendix 3) and record the sign, symptom, or medical diagnosis on the AE eCRF. Additional tests and other evaluations required to establish the significance or etiology of an abnormal result or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the investigator.

8.2.4 Other

[redacted]

8.2.4.2 Pharmacokinetics and Pharmacodynamics

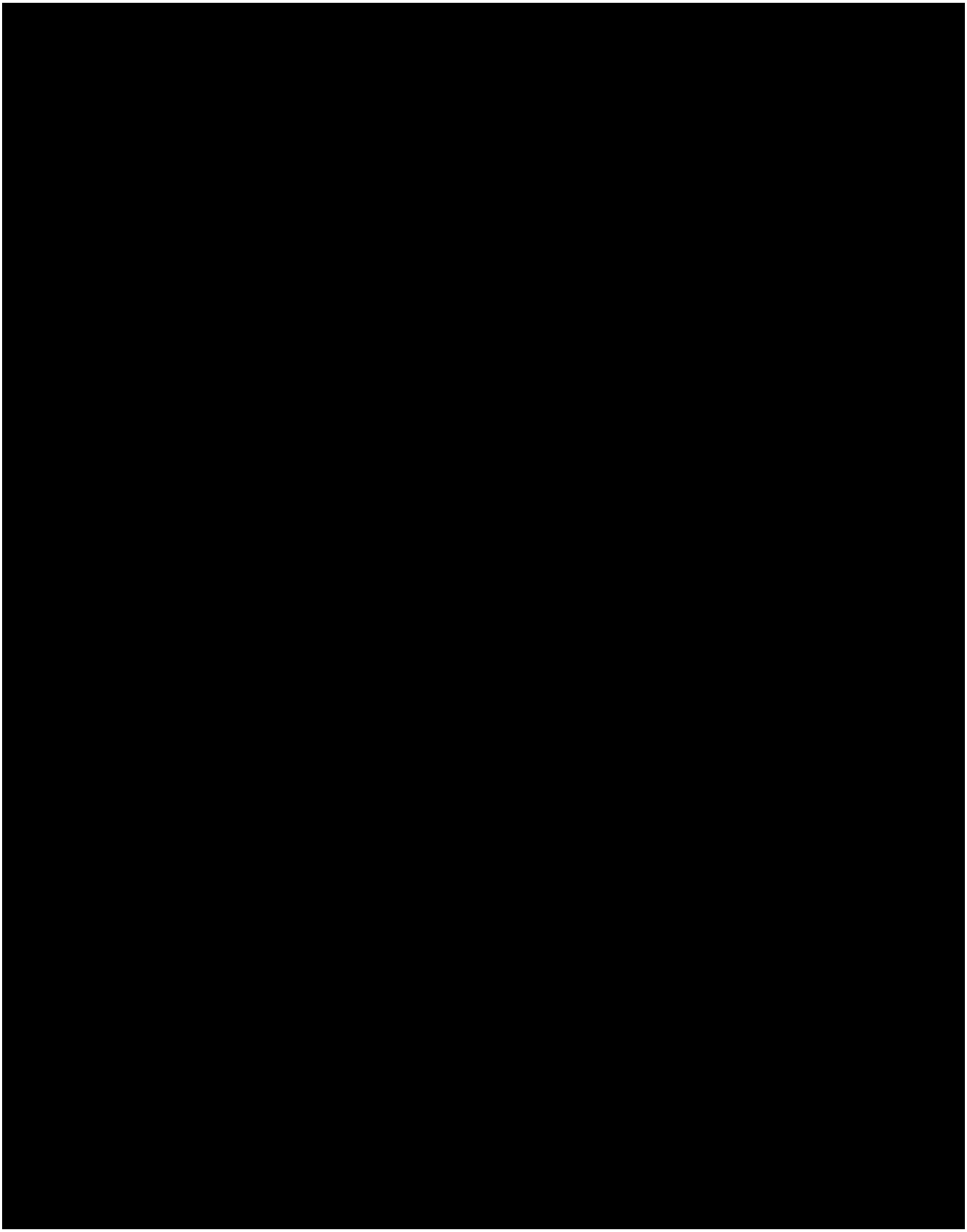
The pharmacokinetic and pharmacodynamic activity of SHP648 will be evaluated using the following assessments:

- FIX activity (central laboratory one-stage clotting assay) and FIX antigen (protein) levels in plasma
- Antibodies to FIX and FIX transgene product
- Neutralizing antibodies against AAV8 and AAV2
- CMI response to AAV8 and FIX transgene product

Graphical displays over time may be used. Pharmacokinetic and pharmacodynamic analyses and modeling may be conducted to assess the timecourse of FIX and potential factors affecting the timecourse of FIX. Analyses and modeling may include, but are not limited to, FIX activity, FIX antigen concentration, clinical chemistry values, vector shedding values, subject demographics, antibody titers, concomitant drug use, clinical measures of effect, and AEs. This optional work will be reported separately.

8.2.4.3 Exploratory Assays

[redacted]



[redacted]

[redacted]

8.2.5 Volume of Blood to Be Drawn from Each Subject

Details of required blood sampling volumes for all mandatory and voluntary tests are presented in the Laboratory Manual and master ICF.

Blood samples that remain after study testing is done may be stored and used to repeat testings, if the first testing does not provide a valid result. These samples will be destroyed at the latest when the final study report is written.

In addition, mandatory backup samples in serum and plasma will be taken and stored short-term. These samples may be used primarily for re-testing and follow-up of an AE(s). Once it becomes apparent that these samples are no longer needed for the above-mentioned purposes, they will be destroyed once the final study report is written unless the patient provided consent that the samples may be used for further research. In this case, the remaining samples may be stored in a coded form for no more than 5 years after the final study report has been completed, and then the samples will be destroyed.

8.2.6 Subject Diary

A paper diary will be provided to each subject at the Screening Visit to record the following information:

1. Details of bleeding episodes.
2. FIX consumption.
3. Concomitant medications and non-drug therapies.
4. Untoward medical occurrences.

For each bleeding episode, the following information will be recorded by the subject or by authorized, qualified personnel at the participating site:

- Location of bleed, e.g., joint, soft tissue, muscle, body cavity, intracranial, other.
- Type of bleed, i.e., spontaneous (definitely no injury/trauma), injury (definitely due to injury/trauma), or unknown.
- Severity of bleed:
  - Minor: Early hemarthrosis, mild muscle bleeding (refer to Appendix 6), or mild oral bleeding, including epistaxis.
  - Moderate: Moderate bleeding into muscles, bleeding into the oral cavity, definite/more extensive hemarthroses, and known trauma.
  - Major, life-/limb-threatening: Significant gastrointestinal bleeding, intracranial, intra-abdominal, or intrathoracic bleeding, central nervous system (CNS) bleeding, bleeding in the retropharyngeal or retroperitoneal spaces or iliopsoas sheath, fractures, head trauma.
- Date and time of onset of bleed.
- Date and time of resolution of bleeding episode.

Subjects will be trained on use of the diary. The diary will be provided in paper format and remain with the subject for the duration of the study. The investigator will review the diary for completeness and request missing information periodically and in a timely manner. Untoward events recorded in the paper diary will be reported as AEs according to the investigator's discretion and clinical judgment. Subject entries in the paper diary will serve as source records.

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Analysis Process

All details regarding the statistical analysis and the preparation of tables, listings, and figures will be described in the statistical analysis plan (SAP). The SAP will provide the statistical methods and definitions for the analysis of the study outcomes, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, investigational product exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

To preserve the integrity of the statistical analysis and study conclusions, the SAP will be finalized prior to database lock. All statistical analyses will be performed using SAS® 9.1 or later version (SAS Institute, Cary, NC 27513).

9.2 Data Monitoring Committee, Adaptive Design and Planned Interim Analysis

An external Data Monitoring Committee (DMC) will be involved in the management of this study. The DMC will provide recommendation for dose escalation decisions as described in Section 6.2.1. Further details regarding the DMC can be found in the DMC charter, which will be available prior to the administration of investigational product.

In addition to safety data reviews by the DMC, additional interim analyses of efficacy and safety data may be performed at study milestones (e.g., when the first two subjects in the first cohort finish the Week 14 visit, or when the first 2 subjects in the last cohort finish the milestone week that will be determined by DMC once two patients in cohort 1 are evaluated for FIX peak expression), or otherwise up to 2 times every year for the duration of the study. Analyses due to different trigger points may be waived or combined for efficiency.

9.3 Sample Size and Power Considerations

The sample size is expected to range from 2 to 21 evaluable subjects depending on the actual number of cohorts and the actual number of subjects in each cohort. This sample size was chosen to provide sufficient evidence of safety and exploration of signs of efficacy for this indication and is not based on formal statistical considerations.

9.4 Statistical Analysis Set(s)

Classification into the Safety Analysis Set will be conducted prior to database lock. The Safety Analysis Set will consist of all subjects who receive any amount of IP. The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline FIX activity assessment. All safety analyses (including the primary analysis) will be performed on the Safety Analysis Set. All efficacy analyses will be performed on the FAS.

9.5 Methods of Analyses

9.5.1 Primary Endpoint

The primary endpoint is the incidence and severity of AEs (serious and non-serious) related to SHP648 that include development of FIX inhibitory antibodies, ECG findings, and clinically significant changes in standard laboratory parameters and in vital signs.

AEs will be coded using the MedDRA. Treatment-emergent AEs (TEAEs) are defined as AEs with start dates at the time of or following the first exposure to IP. The number of events and percentage of TEAEs and AEs related to IP will be tabulated by system organ class, by preferred term, and by treatment cohort. TEAEs and AEs related to IP will be further summarized by severity. AEs leading to withdrawal, SAEs and deaths will be listed.

Clinical laboratory tests, vital signs, and ECG findings will be summarized by cohort and visit. Potentially clinically important findings will also be summarized or listed.

9.5.2 Secondary Endpoints

The secondary endpoints are:

- Circulating plasma FIX activity and FIX antigen levels
- ABR before in comparison to before gene transfer
- Neutralizing and binding antibody titers to AAV8
- T-cell response to AAV8 and FIX transgene products
- Presence of SHP648 genome by type of bodily fluid
- Percentage of change in consumption of exogenous FIX before and after gene transfer

Plasma FIX levels, antigen levels, exogenous FIX consumption, and ABR will be summarized using descriptive statistics by cohort.

The number and proportion of subjects with positive neutralizing and binding antibodies too AAV8, of subjects with T-cell response to AAV8 and FIX transgene, and of those with SHP648 genomes present in bodily fluids after the SHP 648 administration will be summarized by cohort and overall.

9.5.3 Exploratory Endpoints

Exploratory endpoints include

[redacted]

9.6 Other Analyses

No other analyses are planned in this study.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

APPENDIX 1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Appendix 1.1 Regulatory and Ethical Considerations

This study is conducted in accordance with current applicable regulations including ICH E6, EU Directive 2001/20/EC, and all updates, as well as local ethical and legal requirements.

Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the Declaration of Helsinki.

The name and address of each third-party vendor (e.g., CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

Appendix 1.2 Sponsor's Responsibilities

Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, current ICH Good Clinical Practice (GCP) Guidelines, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of investigational product for shipment to the site.

Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

The timing for study registration and results summary posting must be in accordance with applicable local and national requirements.

Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees (ECs)

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.

Appendix 1.3 Investigator's Responsibilities

Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP E6 R2 (2016), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the coordinating principal investigator, in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all investigational product, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC (Institutional Review Board/Ethic Committee), and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

Documentation and Retention of Records

Case Report Forms

In this trial electronic Case Report Forms (eCRFs) will be used.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto CRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. Case report forms must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the CRF.

The CRA/study monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, Health Care Questionnaires, subject diary cards and original clinical laboratory reports.

All key data must be recorded in the subject's source documents.

The investigator must permit authorized representatives of the sponsor; the respective national, local, or foreign regulatory authorities; the IRB/EC; and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the CRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (e.g., subject's medical file, appointment books, original laboratory reports, X-rays etc.).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (e.g., the US FDA, European medicine agency (EMA), UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in investigational product; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

Appendix 1.4 Data Management Considerations

Data Collection

The study will be monitored according to GCP. The investigators' authorized site personnel must enter the information required by the study CRF Completion Guidelines or similar for all data requiring transcription of the source. A study monitor will visit each site in accordance with the monitoring plan and review the CRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the CRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting.

Data Management

Data are to be entered into a clinical database. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database. No changes will be allowed to the source data from patient reported dairies except for the type of data listed below.

List of data that may be modified:

- Demography
- Visit Labels\type
- Site-entered Data
- Data that can be verified through another source system like IRT

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

Data Handling in blinded studies

This paragraph is not applicable to this study.

Appendix 1.5 Ethical Considerations

Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (i.e., a complete set of subject information sheets and fully executed signature pages) must be given to the subject. This document requires translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (i.e., sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

The applicant for an EC opinion can be the sponsor or investigator for sites within the EU; for multicenter studies, the applicant can be the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement. Investigational product supplies will not be released until the CRO has received written IRB/EC approval.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue. If required by local law, substantial amendments to the protocol must also be approved by the appropriate regulatory agency prior to implementation.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol at least annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. This can be the responsibility of the sponsor or investigator for sites within the EU; or for multicenter studies, the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs as required by IRB/EC procedures.

Privacy and Confidentiality

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market SHP648; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities. Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected, if permitted under local laws governing privacy.

The results of studies containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth, where allowed per local law, may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

Study Results/Publication Policy

The term "Publication" shall mean any paper, article, manuscript, report, poster, internet posting, presentation slides, abstract, outline, video, instructional material, presentation (in the form of a written summary), or other public disclosure of the study results, in printed, electronic, oral, or other form. The parties understand and agree that participation in the study may involve a commitment to publish the data from all sites participating in the study in a cooperative publication with other investigators prior to publication or oral presentations of the study results on an individual basis.

The site agrees not to publish or present the site's study results until such time as either the aggregate multi-site study results are published in a cooperative publication or for a period of one (1) year after termination or completion of the study at all participating sites, whichever shall first occur. After that time, the site may publish the site's study results in scientific journals or present the study results at symposia or other professional meetings in accordance with the following provisions:

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single site data being presented.

At least sixty (60) days prior to submitting an abstract, manuscript, or other document for publication, a copy of the proposed publication will be provided to the sponsor by the site for review. Upon the sponsor's request, the site agrees to remove any and all confidential information (expressly excluding study results) identified in the publication and to delay such submission or presentation for an additional sixty (60) day period in order to allow the sponsor time to file any patent application(s). All publications of the study results shall appropriately reference the multi-site study publication, if any, or the fact that the study results are a subset of data resulting from a larger multi-site study.

Shire is committed to transparent dissemination of all scientific, technical and medical manuscripts generated from Shire-supported research. Therefore, after January 1, 2018, Shire will require the submission of all Shire-supported research manuscripts to journals that offer public availability via Open Access (including publisher platforms/repositories and self-archiving). Open Access refers to the free at point of entry, online availability of published research output with, where available, rights of re-use according to an End User License.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) Recommendation for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical journals. Participation as an investigator does not confer any rights to authorship of publications.

APPENDIX 2 CLINICAL LABORATORY TESTS

For a list of the clinical laboratory tests that will be performed and special considerations regarding these laboratory tests, refer to the SHP648-101 Laboratory Manual.

APPENDIX 3 ADVERSE EVENTS: DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

Appendix 3.1 Adverse Event Definitions

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this investigational product or medicinal product. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not causality is suspected (ICH Guidance E2A 1995).

Treatment-emergent Adverse Event

A treatment-emergent adverse event (TEAE) is defined as any event emerging or manifesting at or after the initiation of treatment with an investigational product or medicinal product or any existing event that worsens in either intensity or frequency following exposure to the investigational product or medicinal product.

Serious Adverse Event

A serious adverse event (SAE) is any untoward clinical manifestation of signs, symptoms or outcomes (whether considered related to investigational product or not and at any dose:

- Results in death
- Is life-threatening. Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject 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 was more severe.
- Requires inpatient hospitalization or prolongation of hospitalization. Note: Hospitalizations that are the result of elective or previously scheduled investigations procedures or surgery for pre-existing conditions and have not worsened after initiation of treatment should not be classified as SAEs.
    - For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
    - If the subject requires urgent or emergency surgery after SHP648 administration, it is recognized and expected that during the surgery and in the post-surgical period, the subject can be treated with infused FIX concentrates to achieve FIX levels consistent with the standard of care at the subject institution.
- Results in persistent or significant disability/incapacity

- Results in a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include:
- Bronchospasm associated with anaphylaxis requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.
- Reviewed and confirmed seroconversion for human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), or parvovirus B19 (B19V)
- Development of a confirmed inhibitor to FIX with an inhibitor level ≥0.6 BU using the Nijmegen modification of the Bethesda assay.
- Severe hypersensitivity/anaphylactic reactions to SHP648.

Uncomplicated pregnancies, following paternal exposure to IP are not considered an (S)AE; however, any pregnancy complication or pregnancy termination by therapeutic, elective, or spontaneous abortion shall be considered an SAE.

Any pregnancy that occurs in the partner of the father after administration of IP but prior to clearance of SHP648 genomes from the semen will be reported on a Pregnancy Report Form and followed-up at estimated date of delivery and 1 year post-delivery, if feasible.

For this protocol, bleeding events will not be collected as SAE(s):

- Hospitalization for planned port placement or removal is not considered an SAE, however, any hospitalization required for an emergency port removal is considered an SAE.

Unexpected Adverse Event

An unexpected adverse event is an AE whose nature, severity, specificity, or outcome is not consistent with the term, representation, or description used in the Reference Safety Information (RSI). “Unexpected” also refers to the AEs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the product, but are not specifically mentioned as occurring with the particular product under investigation.

The expectedness of AEs will be determined by the sponsor using the IB as the RSI. This determination will include considerations such as the number of AEs previously observed, but not on the basis of what might be anticipated form the pharmacological properties of a product.

Suspected Unexpected Serious Adverse Reaction

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as any suspected adverse reaction to study treatment (i.e., including active comparators) that is both serious and unexpected.

The event(s) must meet all of the following:

- Suspected adverse reaction
- Serious
- Unexpected
- Assessed as related to study treatment

Symptoms of the Disease under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected disease progression and are part of the efficacy or effectiveness data collected in the study. Significant worsening of symptoms should be recorded as an AE.

For the purposes of this study, the following non-serious events experienced after the first IP exposure are to be collected under other study endpoints and thus are not reportable on the AE eCRF, nor will they be included in the analysis of AEs:

1. Hospital or study site visits for administration of FIX.
2. Hospitalization for routine bleeding episode management that could be managed in the clinic or home-setting but for which the subject was hospitalized.
3. Hospitalizations for planned medical or surgical procedures, e.g., placement of a central venous line (however, when there is an increase in the severity, duration, or frequency of a preexisting disease, the event must be described on the AE eCRF).
4. Seroconversion after documented HAV/HBV vaccination prior to or during the study period.

5. Bleeding episodes/hemophilia-related events:
   Bleeding episodes are part of the underlying disease and therefore are not AEs. If a bleeding episode was caused by an injury (e.g., a fall), the injury would not be reported as an AE, unless it resulted in a medical finding other than a bleeding episode (e.g., abrasion of skin; fractured tibia). Therefore, **any hemophilia-related event** (e.g., hemarthrosis, bruising, hemorrhage) **will not be reported as an AE, but these events will be recorded on the bleeding event eCRF**. However, hemophilia-related events meeting the criteria for seriousness (e.g., a gastrointestinal hemorrhage requiring hospitalization) will be reported as SAEs and described on the SAER form.

Preexisting conditions prior to randomization or initiation of study medication are described in the medical history, and those that manifest with the same severity, frequency, or duration after drug exposure, are not be recorded as AEs. However, when there is an increase in the severity, duration or frequency of a preexisting condition, the event must be described on the AE CRF.

Clinical Laboratory and Other Safety Assessment

A change in the value of a clinical laboratory parameter, vital sign measure, or ECG assessment can represent an AE if the change is clinically relevant or if, during administration of investigational product, a shift of a parameter is observed from a value in the normative range to a value that is outside the normal range and considered clinically significant, or a further waning of an already clinically significant value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing administration or after the end of administration with the investigational product, and the range of variation of the respective parameter within its reference range, should also be considered.

If, at the end of the treatment phase, there are abnormal clinical laboratory (such as hematology panel or clinical chemistry panel), vital sign, or ECG values which were not present at the pretreatment evaluation observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (e.g., concomitant disease or expected disease evolution) is found for the abnormal values.

The investigator should assess, based on the above criteria and the clinical condition of the subject, whether a change in a clinical laboratory value, vital sign, or ECG parameter is clinically significant and represents an AE.

Appendix 3.2 Collection of Adverse Events

All AEs/SAEs are collected from the time the informed consent document is signed until the defined follow-up period stated in Section 8.1.3. This includes events occurring during the screening phase of the study, regardless of whether or not investigational product is administered.

All AEs/SAEs must be followed to closure, regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, or stabilization is achieved (the investigator does not expect any further improvement or worsening of the event). Recovering/resolving AEs will be followed until resolution, medically stabilized, or 4 weeks after the Study Completion/Termination Visit, whichever comes first, and the follow-up information is to be documented in the eCRF.

Appendix 3.3 Assessment of Adverse Events

Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity is captured as a new event. Worsening medical conditions, signs or symptoms present prior to initiation of investigational product, must be recorded as new AEs.

For example, if a subject reports mild intermittent dyspepsia prior to initiation of dosing with the investigational product and the dyspepsia becomes severe and more frequent after first dose, a new AE of severe dyspepsia (with the appropriate date of onset) should be documented in the source.

The medical assessment of severity is determined by using the following definitions:

- Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Relationship Categorization

A physician/investigator must make the assessment of relationship to investigational product for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source.

The following additional guidance may be helpful:

Table A1. Adverse Event Relationship Categorization

Related	The temporal relationship between the event and the administration of the investigational product is compelling enough and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the investigational product and the event.

Outcome Categorization

The outcome of AEs must be documented in the source during the course of the study. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved with Sequelae
- Recovering/Resolving
- Unknown

If applicable, action taken (i.e., dose increased, dose not changed, dose reduced, drug interrupted, drug withdrawn, not applicable, or unknown) will also be recorded on the AE CRF.

Appendix 3.4 Safety Reporting

Reference Safety Information

The RSI for this study is the Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Drug Safety Department and the CRO/Shire medical monitor within 24 hours of becoming aware of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Appendix 3.9) unless they result in an SAE.

The investigator must complete, sign, and date the Shire "Clinical Study Adverse Event Form for Serious Adverse Events (SAEs) and Non-serious AEs as Required by Protocol", verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested), and fax or e-mail the form to the Shire Global Drug Safety Department. A copy of the Shire Clinical Study Adverse Event Form for Serious Adverse Events (SAEs) and Non-serious AEs as Required by Protocol (and any applicable follow-up reports) must also be sent to the CRO/Shire medical monitor using the details specified in the emergency contact information section of the protocol.

Appendix 3.5 Serious Adverse Event Collection Time Frame

All SAEs (regardless of relationship to investigational product) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 8.1.3 and must be reported to the Shire Global Drug Safety Department and the CRO/Shire medical monitor within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the investigational product and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Drug Safety Department within 24 hours of the reported first becoming aware of the event. Follow up and documentation of such SAE(s) will be conducted as per Shire's standard operating procedure.

Appendix 3.6 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms reported by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

Appendix 3.7 Fatal Outcome

Any SAE that results in the subject's death (e.g., the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another investigational product action was previously taken (e.g., drug interrupted, reduced, withdrawn), the action taken with the investigational product should be recorded as "dose not changed" or "not applicable" (if the subject never received investigational product). The investigational product action of withdrawn should not be selected solely as a result of the subject's death.

Appendix 3.8 Pregnancy

All pregnancies are reported from the time informed consent is signed until the defined follow-up period stated in Section 8.1.3.

Any report of pregnancy for the partner of a male study participant must be reported within 24 hours to the Shire Global Drug Safety Department using the Shire Investigational and Marketed Products Pregnancy Report Form.

A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO/Shire medical monitor using the details specified in the emergency contact information section of the protocol

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days and 1 year post-partum.

Pregnancy complications such as spontaneous abortion/miscarriage, elective abortion or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-hCG test or ultrasound result will determine the pregnancy onset date.

Appendix 3.9 Abuse, Misuse, Overdose and Medication Error

Deviations from the protocol-specified dosage, including abuse, misuse, overdose, or withdrawal, medication error (as defined below), failures of expected pharmacological actions, and unexpected therapeutic or clinical benefits will be followed with regard to occurrence of AEs, lack of efficacy, and/or other observations because these events may be reportable to regulatory authorities.

Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of investigational product when used for a non-medical purpose (e.g., to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society
- Misuse – Intentional use of investigational product other than as directed or indicated at any dose (Note: this includes a situation where the investigational product is not used as directed at the dose prescribed by the protocol)
- Overdose – Intentional or unintentional intake of a dose of investigational product higher than the protocol-prescribed dose
- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an investigational product. For studies, medication errors are reportable to the sponsor only as defined below.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of an expired investigational product should be considered as a reportable medication error.

Appendix 3.10 Urgent Safety Measures

An urgent safety measure is an immediate action taken, which is not defined by the protocol, in order to protect subjects participating in a clinical trial from immediate harm, these do not constitute de facto deviation from the protocol. Urgent safety measures may be taken by the sponsor or clinical investigator, and may include any of the following:

- Immediate change in study design or study procedures
- Temporary or permanent halt of a given clinical trial or trials
- Any other immediate action taken in order to protect clinical trial participants from immediate hazard to their health and safety

The investigator may implement urgent safety measures to protect study subjects from immediate hazard to their health or safety. The measures should implement immediately and does not require prior authorization from the sponsor. In the event(s) of an apparent direct hazard to the subject, the investigator will notify the sponsor immediately by phone and confirm notification to the sponsor in writing as soon as possible, and within 1 calendar day after the change is implemented. The sponsor will also ensure the responsible EC(s) and relevant competent authority(s) are notified of the urgent safety measures taken in such cases according to local regulations.

Appendix 3.11 Regulatory Agency, Institutional Review Board, Ethics Committee, Data Monitoring Committee and Site Reporting

The Sponsor and the clinical CRO are responsible for notifying the relevant regulatory authorities and central ECs of related, unexpected SAEs.

In addition, the sponsor is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP648 program.

The investigator is responsible for notifying the local IRB/EC of SAEs or significant safety findings that occur at his or her site as required by IRB/EC procedures (see Appendix 1.5).

This study will be monitored by a DMC. Enrollment and escalation to the next dose cohort will be paused until an independent DMC evaluates all available study data and makes a recommendation if anyone of the following criteria is met:

- A value of 3x ULN or greater in ALT, AST, or both in any subject after SHP648 administration that is not responsive after 12 weeks of corticosteroid rescue treatment.

- A SAE that may be potentially related to SHP648 and which poses either an immediate risk to the subject's health or is likely to adversely affect the subject's health long term. This includes events classified as AEs qualifying for special notification (Brussels, 03/12/2009, ENTR/F/2/SF/dn D(2009) 35810; EMEA/CHMP/GTWP/60436/2007), if these are judged as potentially related to SHP648.
- The development in any subject of an inhibitor towards FIX (or FIX Padua) after having received SHP648. Further investigations of the characteristics and potential contributing factors and causal relationships of the observed FIX (or FIX Padua) inhibitor will be initiated.
- Death of subject after having received SHP648, that is judged as, definitely, probably or possibly attributed to SHP648. Enrollment in the study and further dosing will be temporarily stopped in order to undergo review by the applicable regulatory authorities and the DMC.
- Occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to SHP648.
- Occurrence of moderate or severe-drug-related AEs in 2 or more SHP648 treated subjects in a given cohort.
- If any other drug-related event occurs in SHP648 treated subjects and is deemed to pose an unacceptable risk to subjects by the investigator or medical monitor after further evaluation, additional subjects will not be enrolled or dosed until a decision is taken to stop or proceed with the study based on further evaluation of the available data by the DMC.

APPENDIX 4 CONTRACEPTIVE GUIDANCE

Male participants:

Male participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the following in the period from SHP648 administration and until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least two consecutive laboratory values for vector genome in semen samples are negative:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year when having penile-vaginal intercourse with a partner of childbearing potential who is not currently pregnant

Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration in the period from SHP648 administration and until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least two consecutive laboratory values for vector genome in semen samples are negative.

Sexually active man must agree to use a condom during sexual intercourse or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners in the period from SHP648 administration until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least 2 consecutively collected semen samples assessed at the central laboratory (this criterion is applicable also for subjects who are surgically sterilized).

Refrain from donating sperm in the period from SHP648 administration and until AAV8 has been cleared from semen, as evidenced from negative analysis results for at least two consecutive laboratory values for vector genome in semen samples are negative.

APPENDIX 5 GENETICS

Shire intends to apply genetic research across the SHP648 development program to explore how genomic variations may affect the clinical parameters associated with and response to SHP648 (and any background products, comparators, and concomitant medications), and potentially the basis of the indications under study in the protocol, in this case hemophilia B. Collection of appropriate samples from populations with well described clinical characteristics may lead to improvements in the design and interpretation of clinical studies, genetically guided treatment strategies, and a better understanding of disease etiology, which may lead to new therapeutic approaches.

Candidate genes which may be studied include those potentially related to the mechanism of action of SHP648 as well as those potentially responsible for absorption, disposition, metabolism, and excretion of SHP648. Future research may suggest other genes, gene categories, proteins, etc., as candidates for influencing not only response to SHP648 and, but also susceptibility to hemophilia B for which SHP648 may be evaluated. Thus, this additional genomic research may involve the future study of additional unnamed genes or gene categories, but only as they relate to hemophilia B disease susceptibility and drug action.

Samples will be drawn at the Screening Visit (3mL blood samples) for whole exome sequencing. Samples may only be collected from subjects who provide separate informed consent.

Samples will be labeled with the study protocol number, the subject's study identification number, and information related to the sample. No personal identifiers will be recorded on the sample labels.

Subjects terminating early from the study due to AE, tolerability, or drug-related issues should, where possible, be approached for their remaining protocol-defined samples at the earliest possible time. Unscheduled samples should be labeled with free text capturing study protocol number, subject's study identification number, and information related to the sample (RNA or protein, sampling date, and time). Samples will be shipped to and stored at biorepositories as detailed in the laboratory manual. [redacted]

As an added level of security, the sample will be recoded with a new, unique number at the biorepository laboratory. This unique number is the only code used in any subsequent analysis and will be used to link a sample to a subject and to ensure that the subject's identity remains confidential.

A link file linking the first and second codes will be kept in a secure place at the sponsor, with restricted access. This will be in a secure environment outside of the clinical study database and separate to any analysis results. This file will be used to identify the relevant samples for analysis, facilitate correlation of any results with clinical data, allow regulatory audit, and trace samples for destruction in the case of withdrawal of consent. No record of participation in this pharmacogenomics portion of the protocol, or any results derived from it, should be recorded in the subjects' personal medical records. A record of participation in the pharmacogenomics portion of the protocol will, however, be captured in the study-specific source documentation records or CRF.

The sponsor, sponsor's representatives, biorepositories, and any specialty laboratories will be blinded to the subject's identity. The sample and/or extracted material will otherwise be stored for up to 5 years from the end of the study after which time it will be destroyed. Upon written request, subjects will be permitted to withdraw their sample from the analysis and have their sample and/or extracted material destroyed. The link will also be destroyed at the same time as any remaining sample(s) are destroyed. Any results already generated from the samples will not be removed from any analyses that have already been performed.

Participation in this portion of the study is optional and does not impact the subject's eligibility for participation in the main clinical study. Subjects may continue to participate in the primary study if they refuse to provide a blood sample or if they withdraw their samples.

[redacted]

APPENDIX 6 BLEED DEFINITIONS

Appendix 6.1 Joint Bleeds

Features of an acute joint bleed include some or all of the following: 'aura', pain, swelling, warmth of the skin over the joint, decreased range of motion and difficulty in using the limb compared with baseline or loss of function.

The earliest clinical signs of a joint bleed are increased warmth over the area and discomfort with movement, particularly at the ends of range.

Later symptoms and signs include pain at rest, swelling, tenderness, and extreme loss of motion.

In patients with advanced arthropathy it may be difficult to distinguish pain-related arthritis from that associated with an acute bleed. Rapid resolution of pain following infusion of factor concentrates (typical of an acute hemarthrosis) or improvement of pain associated with activity soon after a period of rest (typical of chronic arthritis) can help distinguish between the two.

In infants and young children, reluctance to use the limb alone may be indicative of a joint/muscle bleed.

Appendix 6.2 Muscle Bleeds

Muscle bleeds can occur in any muscle of the body, usually from a direct blow or a sudden stretch. A muscle bleed is defined as an episode of bleeding into a muscle, determined clinically and/or by imaging studies, generally associated with pain and/or swelling and functional impairment over baseline.

For further definitions of central nervous system (CNS), gastrointestinal, and abdominal hemorrhages see the Guidelines for the Management of Hemophilia from the World Federation of Hemophilia (World Federation of Hemophilia Treatment Guidelines Working Group, 2012).

Appendix 6.3 Target Joints

A target joint is defined as a joint with three or more bleeds within a period of six consecutive months.

APPENDIX 7 ABBREVIATIONS

Abbreviation	Definition
AASLD	American Association for the Study of Liver Disease
AAV	Adeno-Associated Virus
AAV2	AAV of serotype 2
AAV8	AAV of serotype 8
AE	adverse event
ABR	Annualized bleed rate
ALT	alanine aminotransferase
ANA	Antinuclear antibody
AST	aspartate aminotransferase
BU	Bethesda unit
BGH polyA	bovine growth hormone polyadenylation sequence
BW	body weight
CD4	cluster of differentiation 4
CMI	cell-mediated immune
CpG	5' cytosine – phosphate – guanine 3'
cp	capsid particles
CRM 8	liver-specific cis-regulatory module
CRO	contract research organization
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
EASL	European Association for the Study of the Liver
ECG	Electrocardiogram
EC	Ethic Committee
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicine Agency
[redacted]	[redacted]
FAS	full analysis set
FDA	US Food and Drug Administration
F8	human gene encoding coagulation factor VIII
F9	human gene encoding coagulation factor IX
FVIII	factor VIII
FIX	factor IX

Abbreviation	Definition
FIX_R338L	factor IX with arginine at position 338 replaced by a lysine (Padua mutation)
GCP	good clinical practice
[redacted]	[redacted]
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
[redacted]	[redacted]
HIV	Human Immunodeficiency Virus
[redacted]	[redacted]
[redacted]	[redacted]
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IB	Investigator's Brochure
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
ITR	inverted terminal repeat
IU	international Units
IV	intravenous(ly)
kg	kilogram
LKM1	anti-liver-kidney microsomal antibody type 1
mg	milligram
mL	milliliter
mM	Millimolar
MVM	Minute Virus of Mice
NOAEL	No observed adverse effect level
PAMPs	Pathogen Associated Molecular Patterns
PCR	polymerase chain reaction
Q3	third year quartal
rAAV	recombinant AAV
rep	replication
RNA	ribonucleic acid
RSI	reference safety information
SAE	serious adverse event

Abbreviation	Definition
SAP	statistical analysis plan
[redacted]	[redacted]
SHP648	Shire FIX gene therapy product (AAV8.ss-3xCRM8-TTR-FIX_R338Lopt), also known as TAK748
SIC	subject identification code
ss	single-stranded
SUSAR	suspected unexpected serious adverse reaction
TTR	transthyretin
ULN	upper limit of normal
vg	vector genome
VP	viral structural protein
WT	wild type

APPENDIX 8 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Amendment 1.0	21 FEB 2020	Global
Original Protocol	13 MAY 2019	Global

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