Clinical Study Protocol

NCT Number: NCT03370172

Title: A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion

Study Number: 201501

Document Version and Date: Amendment 9, 10 NOV 2021

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

PROTOCOL: 201501

TITLE:	A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion
SHORT TITLE:	Safety and Dose Escalation Study of an Adeno-Associated Viral Vector for Gene Transfer in Hemophilia A Subjects
STUDY PHASE:	*Phase 1/2*
DRUG:	BAX 888
IND NUMBER:	017523
EUDRACT NUMBER:	2015-005576-22
NCT NUMBER:	NCT03370172
SPONSOR:	Takeda Development Center Americas, Inc.* 95 Hayden Ave, Lexington, MA 02421 USA AND Baxalta Innovations GmbH** Industriestrasse 67, A-1221 Vienna AUSTRIA *Formerly Baxalta and later Shire **Baxalta is now part of Takeda
PROTOCOL HISTORY:	**Amendment 9: 2021 NOV 10** Replaces: **Amendment 8: 2021 MAR 22** Amendment 7:2020 MAR 12 Amendment 6: 2019 MAY 03 Amendment 5: 2018 AUG 16 Amendment 4: 2018 JAN 24 Amendment 3 (USA): 2017 SEP 01 Amendment 2 (USA): 2017 AUG 03 Amendment 1: 2017 MAR 15 Original: 2016 FEB 01

PROTOCOL SIGNATURE PAGE

Sponsor's (Takeda) Approval

Signature: [redacted]	**Date:** 11-Nov-2021 | 09:57:49 JST
[redacted] M [redacted]	

Investigator's Acknowledgement

I have read this protocol for Study 201501.

Title: A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion

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:** _____

1. STUDY PERSONNEL

1.1 Authorized Representative (Signatory) / Responsible Party

[redacted], PhD

[redacted] GT/Hematology

1.2 Study Organization

The name and contact information of the responsible party and individuals involved with the study (eg, investigator[s], sponsor's medical expert and study monitor, sponsor's representative[s], laboratories, steering committees, and oversight committees (including ethics committees [ECs], as applicable) will be maintained by the sponsor and provided to the investigator.

2. SERIOUS ADVERSE EVENT REPORTING

The investigator will comply with applicable laws/requirements for reporting serious adverse events (SAEs) to the ECs.

ALL SAEs, INCLUDING SUSARs, ARE TO BE REPORTED ON THE SERIOUS ADVERSE EVENT REPORT (SAER) FORM AND TRANSMITTED TO THE SPONSOR WITHIN 24 HOURS AFTER BECOMING AWARE OF THE EVENT

Drug Safety contact information: see SAE Report form.
Refer to SAE Protocol Sections and the study team roster for further information.

For definitions and information on the assessment of these events, refer to the following:

1. Adverse events (AEs), Section 11.1
2. SAE, Section 11.1.1.1
3. SUSARs, Section 11.1.1.2
4. Assessment of AEs, Section 11.1.2.

3. SYNOPSIS

INVESTIGATIONAL PRODUCT	
Name of Investigational Product (IP)	BAX 888
Name(s) of Active Ingredient(s)	AAV8.BDD-FVIIIopt
CLINICAL CONDITION(S)/INDICATION(S) • Severe hemophilia A (≤1% Factor VIII [FVIII] activity)	
PROTOCOL ID	201501
PROTOCOL TITLE	A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion
Short Title	Safety and Dose Escalation Study of an Adeno-Associated Viral Vector for Gene Transfer in Hemophilia A Subjects
STUDY PHASE	Ph1/2
PLANNED STUDY PERIOD	
Initiation	Q1 2018 (First subject first visit)
Primary Completion	Q3 2021 (Last subject in)
Study Completion	Q3 2026 (Last subject out)
Duration	Approximately 8 years
STUDY OBJECTIVES AND PURPOSE	
Study Purpose 1. To evaluate the safety and determine the dose of BAX 888 required to achieve FVIII activity levels ≥20% of normal in severe hemophilia A subjects.	
Primary Objective 1. To evaluate the safety of a single intravenous (IV) infusion of BAX 888 in up to 3 dose cohorts.	
Secondary Objectives 1. To evaluate plasma FVIII activity levels pre- and post-BAX 888 infusion and investigate the relationship between FVIII activity levels and BAX 888 dose. 2. To determine the BAX 888 dose needed to achieve FVIII activity level of ≥20% of normal in ≥60% of the subjects. 3. To collect bleeding rate and consumption of exogenous FVIII after gene transfer. 4. To assess humoral and cellular immune responses to FVIII and the AAV8 viral capsid. 5. To determine the duration of BAX 888 genome presence in blood, saliva, semen, urine and stool.	

STUDY DESIGN	
Study Type/ Classification/ Discipline	Safety, Efficacy, Pharmacodynamics
Control Type	No control
Study Indication Type	Treatment
Intervention model	Single-administration, up to 3 cohorts
Blinding/Masking	Open-label
Study Design	This is a global, Phase 1/2 multicenter, open-label, safety and dose escalation study of BAX 888 in adult subjects with severe hemophilia A. Up to 12 subjects may be administered BAX 888 in up to 3 dose cohorts. **<u>Study Procedures</u>** Before any study-related procedures are followed, subjects will provide signed informed consent[i]. During the course of the study, FVIII activity levels will be assessed by a central laboratory (both one-stage and chromogenic assays) and by the local laboratory (either one-stage or chromogenic assay) as specified in Table 3. However, only central laboratory FVIII activity levels derived from the one-stage assay will be utilized for the primary analysis. FVIII activity levels obtained from the central laboratory chromogenic assay will be utilized to support the one-stage assay results. Baseline FVIII activity levels will be assessed by the central laboratory one-stage clotting assay at the Screen 1 visit. Upon completion of screening activities, on the day of BAX 888 dosing (Day 0)[ii], subjects will be admitted to the infusion center[iii]. BAX 888 will be administered as a single peripheral IV infusion on Day 0. Subjects will be evaluated at the infusion center for the first 8 hours post-BAX 888 infusion (and up to 24 hours at the discretion of the investigator), and for the Day 1 visit. The subject will then be evaluated at the hemophilia treatment center at Weeks 1 and 2 (once weekly), Weeks 3 to 14 (twice weekly, with separate clinic visit and laboratory visit each week), Weeks 15 to 18 (once weekly), and at Months 5, 6, 9, 12, 16, 20, 24, 28, 32, 36, 48, and 60. To assess long-term safety (adverse events [AEs]) and efficacy (FVIII activity levels, bleeding episodes, FVIII consumption) subjects will continue to be followed every 4 months through Years 2 and 3, and annually on Years 4 and 5. Blood samples and urine for clinical safety evaluations will be collected and FVIII activity will be collected as an efficacy-related parameter to support future clinical

[i] Subjects will initially provide consent for the Screen 1 visit. Subjects who pass Screen 1 will then provide consent for the second screening visit and all subsequent study visits at the Screen 2 visit.

[ii] If the infusion center is not the subject's Hemophilia Treatment Center/local investigator's institution, a second separate consent will be obtained from the subject for the Day 0 visit and for all procedures surrounding the infusion period.

[iii] At the discretion of the investigator, subjects may remain in the infusion center for 24 hours following infusion, or return to the center for follow-up at 24 hours post-infusion.

	development. Electrocardiograms will be obtained, and vital signs will be monitored frequently over the first 8 hours following BAX 888 infusion. Blood and bodily secretions (saliva, semen, urine, and stool) will be obtained to assess BAX 888 genomes until 2 consecutive measurements are negative. In addition to a safety data review by the Data Monitoring Committee (DMC), further analyses on efficacy and safety data may be performed at study milestones (eg, when the initial two subjects in the first cohort finish the Week 18 visit), or otherwise up to twice a year for the duration of the study. Analyses due to different trigger points may be waived or combined for efficiency. Clinical bleeding episodes will be managed with infusion of exogenous FVIII. Usage of exogenous FVIII for prevention (prophylactic treatment) or episodic management (on-demand 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 measure FVIII activity that is derived from the BAX 888 transgene and not exogenous FVIII products, subjects will be asked to refrain from prophylactic FVIII usage between Weeks 3 to 18 after BAX 888 administration and during the 3 weeks prior to the Month 9 visit. During these periods which are critical for the assessment of sustained FVIII activity levels 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 FVIII product (rather than an extended half-life FVIII concentrate). Whenever possible, a 3- to 5-day wash-out period should be observed prior to any study visit with a FVIII activity level assessment, however, scheduled visits (except for Screen 1) should not be missed in the case of a wash-out of less than 3 days. **Dose Escalation** At study initiation, subjects will be assigned to a dose cohort, with a minimum of 24 hours between dosing of each subject. Initially, 2 subjects will be dosed in a cohort, with up to a total of 5 subjects if the cohort is expanded. Safety and FVIII activity level data from subjects dosed with BAX 888 will be utilized to make decisions on dose escalation and cohort expansion. Rules for cohort expansion and dose escalation are provided below. However, if any subject in a given cohort achieves >50% FVIII activity levels at Week 14, the DMC will provide a recommendation on further dose escalations after review of all safety and FVIII activity level data[iv]. If any subject in any cohort achieves ≥150% FVIII activity levels at any time while on the study, further dosing will be paused until DMC review. Baseline FVIII activity level is defined as the value obtained based on the central laboratory one-stage FVIII assay.

[iv] DMC recommendations will be based on, but not limited to, the following considerations: (1) mean, median, and range of FVIII activity levels (central lab, one-stage clotting assay) observed within the dosing cohort, (2) variability in response between subjects, (3) liver function tests, and (4) other product-related adverse events (AEs)

	Only central laboratory one-stage FVIII activity levels will be considered for dose escalation or cohort expansion decisions. Subjects within the same dose cohort will be infused with BAX 888 no less than 24 hours apart to ensure sufficient time is given to observe for infusion-related hypersensitivity reactions prior to dosing the next subject. Since asymptomatic liver inflammation has not been observed in any individuals who have not expressed FIX or FVIII in the AAV hemophilia gene therapy trials, hence dosing of subjects after dose escalation can occur as early as 4 weeks in the absence of FVIII expression in the lower dose subjects. **Cohort 1 (2.0×10^{12} cp/kg):** After dosing of the first 2 subjects in Cohort 1: • If Week 4 FVIII activity levels of both subjects are <2%, then dose escalation to Cohort 2 will be triggered with no further dosing in Cohort 1. Alternatively, if FVIII activity levels (≥2%) are observed in at least 1 subject, the decision to escalate dose or expand the cohort will be based on all data available through Week 14. • If sustained Week 14 FVIII activity levels of ≥30%[v] are not achieved in both subjects, then escalation to Cohort 2 will be triggered after DMC review of all available safety and FVIII activity level data inclusive of Week 14. • If sustained Week 14 FVIII activity levels of ≥30%v are achieved in at least 1 of the 2 subjects, then expansion of Cohort 1 will be initiated with dosing of 3 additional subjects. <u>For an expanded Cohort 1 with 5 subjects dosed with BAX 888:</u> • If sustained Week 14 FVIII activity levels of ≥30%[v] are not achieved in at least 3 of the 5 subjects, then escalation to Cohort 2 will be triggered after DMC review of all available safety and FVIII activity level data inclusive of Week 14. • If sustained Week 14 FVIII activity levels of ≥30%[v] are achieved in at least 3 of the 5 subjects, one of the following would be pursued based on the safety, FVIII activity levels, and the variability in response that is observed among the 5 dosed subjects: ➢ Completion of study enrollment with no further dosing of additional subjects since the secondary objective to determine a BAX 888 dose needed to attain FVIII activity levels ≥20% in at least 60% of the subjects would have been achieved.

[v] Sustained Week 14 FVIII level ≥30% is defined as 3 or more FVIII activity level measurements ≥30% between Week 12 and Week 18. If applicable per protocol, a DMC meeting will be held when either 3 or more FVIII activity level measurements ≥30% between Weeks 12 and Week 18 are obtained or after Week 18, whichever comes first.

- Expansion of Cohort 1 to include dosing of additional subjects at the same dose level.
- Escalation to a higher dose (eg, subsequent patients will be enrolled in Cohort 2).

Cohort 2 (6.0×10^{12} cp/kg):

After dose escalation and administration of BAX 888 to the first 2 subjects in Cohort 2:

- If sustained Week 14 FVIII activity levels of ≥30%[v] are not achieved in both subjects, then expansion of Cohort 2 or escalation to Cohort 3 will be triggered after DMC review of all data inclusive of Week 14[iv].
- If sustained Week 14 FVIII activity levels of ≥30%[v] are achieved in at least 1 of the 2 subjects, then, after review of all the available data the DMC will make a recommendation to continue with expansion of Cohort 2 up to 5 subjects, or escalate dosing to Cohort 3 (1.2×10^{13} cp/kg).

<u>For an expanded Cohort 2 with up to 5 subjects dosed with BAX 888:</u>

- If sustained Week 14 FVIII activity levels of ≥30%[v] are not achieved in at least 3 subjects, then one of the following would be pursued after DMC review of all available safety and FVIII activity level data inclusive of Week 14:
  - Escalation to Cohort 3 (1.2×10^{13} cp/kg)
  - Completion of study enrollment with no further dosing
- If sustained Week 14 FVIII activity levels of ≥30%[v] are achieved in at least 1 of the subjects, one of the following would be pursued after DMC review and based on the safety, FVIII activity levels, and the variability in response, and other clinical data that is observed among all the dosed subjects in the study:
  - Continue to expand Cohort 2 up to 5 subjects
  - Escalation to Cohort 3 (1.2×10^{13} cp/kg)
  - Completion of study enrollment with no further dosing

Preliminary evaluation following 2 subjects dosed with 2.0×10^{12} cp/kg (lowest dose level) showed minimal impact on FVIII activity levels. Preliminary evaluation following 2 subjects dosed 6.0×10^{12} cp/kg (middle dose level; 3-fold higher than lowest dose level) suggests that the peak FVIII activity was as expected but increased more than proportional to the increase in dose. A mouse model suggested a potential non-linear dose-FVIII activity relationship (doubling of the dose might produce more than a doubling of the FVIII activity). To ensure informed dose escalation in subjects with Hemophilia A, instead of a 3-fold increase in dose, the next proposed dose is 1.2×10^{13} cp/kg which is 2-fold the middle dose level originally proposed.

Cohort 3 (1.2×10^{13} cp/kg)

After dose escalation and administration of BAX 888 to the first 2 subjects in Cohort 3

- If sustained Week 14 FVIII activity levels of ≥30%v are not achieved in both subjects, then dosing of additional subjects will be paused until further review of all available data.
- If sustained Week 14 FVIII activity levels of ≥30%[v] are achieved in at least 1 of the 2 subjects, then expansion of Cohort 3 will be initiated with dosing of up to 3 additional subjects.
- Study could be completed with no further dosing.

Immunosuppression

Vector-derived factor expression followed by a decrease in factor levels has been observed in previous hemophilia gene therapy trials and has been attributed to an immune response directed against the successfully transduced cells. To address this issue and to ensure sustained factor levels are achieved, BAX 888 has been engineered to have a reduced prospect of eliciting an immune response.

Nevertheless, a prophylactic course of glucocorticosteroid treatment with prednisolone (as shown in the table below) will be employed starting at Day 8 post-BAX 888 infusion.

The following dosing schedule will be followed as a guideline. The corticosteroid regimen may be adjusted at the discretion of the Investigator in consultation with the Sponsor Medical Monitor depending on the subject's tolerance of the regimen and the observed hepatic transaminase response.
Subjects receiving corticosteroids may require additional unscheduled visits to evaluate potential side effects of corticosteroid therapy and for corticosteroid dose adjustments, FVIII activity levels and liver function tests (LFTs) will be measured at these unscheduled visits by local laboratories.

Prednisolone Dosing Regimen

Prednisolone Dose (mg/day)	Duration
60	4 weeks
40	2 weeks
30	2 weeks
25	2 weeks
20	2 weeks
15	1 week
10	1 week
5	1 week

Determine alanine aminotransferase (ALT) and FVIII activity level weekly or twice weekly (as per study protocol).

	NOTE: In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (eg USA), prednisone will be provided. Prednisone is converted to the active metabolite prednisolone and is recommended by 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. Assessment of possible reactions to corticosteroids will be performed by the Investigator and modification of treatment will be done as necessary after consultation with the Sponsor's Medical Monitor. In such instances, consideration should be given to combination therapy with azathioprine/corticosteroid per AASLD guidelines or other drug as per standard of practice, in consultation with a hepatologist, so that the care can be individualized to the subject's needs. **Further Management of Presumptive Vector-Related Hepatitis** Any subject who develops an ALT and/or or aspartate aminotransferase (AST) level >3 × upper limit of normal (ULN) during the study will continue to be treated with prednisolone[vi] as outlined in the Prednisolone Dosing Regimen Table above in the Immunosuppression Section. Additionally, the subject must have the test repeated and confirmed within 72 hours. If the level remains >3 × ULN, a diagnostic workup of potential infectious and toxic/metabolic causes of hepatitis will be pursued, including consultation with a hepatologist. If no other cause of the elevated ALT and/or AST is identified, consideration should be given to combination therapy with azathioprine/corticosteroid per AASLD guidelines or other drug regimens as per standard of practice, in consultation with a hepatologist. Recurrent elevations in transaminases after Year 1 should be evaluated and treated at the discretion of the investigator. Abdominal ultrasound examination will be performed locally to assess liver health. In addition, and at the discretion of the Investigator serum alpha fetoprotein level, an exploratory biomarker for hepatocellular carcinoma, will be measured at local laboratories. **Study Stopping Rules** Each subject will receive only 1 dose of BAX 888 during the course of the study; hence study stopping rules apply only to subjects during the screening period. Subjects who have been dosed with BAX 888 will be followed to the end of the study as per protocol. If any one of the following criteria are met, dosing will be paused and further enrollment will be halted until an independent DMC evaluates all available study data and makes a recommendation:

[vi] In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (eg, 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.

	• More than 3 × ULN increase in ALT, aspartate aminotransferase (AST), or both in any subject after BAX 888 administration that is not responsive after 12 weeks of corticosteroid rescue treatment implemented according to AASLD guidelines. • A serious adverse event (SAE) that may or may not be potentially related to BAX 888 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 the investigational product (IP). • The development, after having received BAX 888, of an inhibitor towards FVIII in any subject will lead to pausing of dosing and further investigations of the characteristics and potential contributing factors and causal relationships of the observed FVIII inhibitor. • Death of a subject, after having received BAX 888, that is judged as definitely, probably or possibly attributed to BAX 888. The study will be temporarily stopped in order to undergo review by the institutional review board (IRB), DMC and the FDA. • The occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to BAX 888. Enrollment in the study may be terminated if one or more of the following criteria are met: • The sponsor decides to stop enrollment into the study based upon its assessment of safety. • The sponsor decides to stop enrollment into the study for administrative reasons. If enrollment on the study is terminated, the study will remain open for clinical safety evaluations and FVIII activity level assessments as an efficacy-related parameter that will be recorded for approximately the first five years post subject dosing.
Planned Duration of Subject Participation	The overall duration of the study is approximately 8 years from study initiation (ie, first subject enrolled) to study completion (ie, last subject last visit, 5 years post-gene transfer). The recruitment period is expected to be about 40 months. Each subject participation period is expected to be up to 5.5 years. Every effort will be made to follow subjects for approximately 5-years post-gene transfer period.
Primary Outcome Measure 1. Incidence of BAX 888-related AEs (serious or non-serious) including development of FVIII inhibitory antibodies, clinically significant changes in standard laboratory parameters, physical exam, and vital signs that are reported as AEs.	

Secondary Outcome Measures

Efficacy

1. Circulating plasma FVIII activity and antigen levels.
2. Annualized bleed rate (ABR) in comparison to before gene transfer.
3. Consumption of exogenous FVIII in comparison to before gene transfer.

Safety

1. Development of inhibitory and total binding antibodies to FVIII.
2. Humoral and cell-mediated immune response to AAV8 and FVIII proteins.
3. Surveillance of AAV8 genome shedding in blood, saliva, semen, urine and stool.

Tertiary Outcome Measures

1. Hemophilia Joint Health Score at screening and Months 12, 24, 36, 48, and 60 post-gene transfer.
2. Changes in the following assessments:
   - Health-related quality of life (HRQoL) (post-gene transfer vs. baseline) measured by:
     - Generic: 36-item Short Form survey (SF-36v2), EuroQol-5D (EQ-5D).
     - Disease-specific: Hemophilia-Specific Quality of Life Index (Haemo-QoL-A).
   - Patient experience (baseline):
     - Adherence: Validated Hemophilia Regimen Treatment Adherence Scale-Prophylaxis (VERITAS-Pro).
     - Disease-specific patient experience: Patients' preference for hemophilia treatment questionnaire (HaemoPREF).

INVESTIGATIONAL PRODUCT(S), DOSE AND MODE OF ADMINISTRATION

Active Product	BAX 888 (AAV8.BDD-FVIIIopt) **Dosage form**: Injection, solution. **Dosage frequency**: Once. No repeat dose will be given to same subject. **Mode of Administration:** IV infusion.

SUBJECT SELECTION

Targeted Accrual	Up to 12 evaluable subjects.
Number of Groups/ Arms/ Cohorts	Up to three dose cohorts.

Inclusion Criteria

Subjects must meet all of the following criteria prior to enrollment into the study:

1. Male, aged 18 to 75 years at the time of screening.
2. Established severe hemophilia A (FVIII:C <1%, measured following ≥5 days without FVIII treatment) **and/or** documented intron 1 inversion or intron 22 inversion mutation in the *F8* gene, consistent with severe hemophilia A **AND** documented evidence of ≥3 hemorrhages over the previous 12 months requiring treatment with exogenous FVIII **or** use of FVIII prophylaxis because of history of frequent bleeding episodes
3. History of >150 exposure days to exogenously administered FVIII concentrates or cryoprecipitate.

4. Sexually active men must agree to use barrier contraception (combination of a condom and spermicide) or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners for a minimum of 6 months after administration of BAX 888, or until BAX 888 genomes are no longer detected in the semen, whichever is sooner.
5. Subject is willing and able to comply with the requirements of the protocol, including provision of semen samples, maintenance of a diary of bleeding episodes and FVIII protein use.
6. Signed informed consent.

Exclusion Criteria

Subjects meeting any one of the following criteria will be deemed ineligible for participation in the study:

1. Bleeding disorder(s) other than hemophilia A.
2. Personal laboratory evidence of having developed inhibitors to FVIII protein at any time (≥0.6 Bethesda Units on any single test).
3. Documented prior allergic reaction to any FVIII 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 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, difficult to control hypertension and difficult to control diabetes).
7. Evidence of markers of potential underlying risk for autoimmune mediated hepatic disease:
   a. Anti-smooth muscle antibody assay results ≥40 (Inova QUANTA Lite™ Actin IgG enzyme-linked immunosorbent assay); values of 31 to 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 immunoglobulin G (IgG) >1.5 × ULN.
   d. Antinuclear antibody (ANA) titer >1:320; OR ANA titer >1:80 if demonstrated concurrently with ALT that is >ULN.
8. Active Hepatitis C virus (Hepatitis C): As indicated by detectable HCV ribonucleic acid by polymerase chain reaction.
9. Hepatitis B: If surface antigen is positive.
10. Seropositive for Human Immunodeficiency Virus (HIV)
11. Receiving systemic antiviral and/or interferon therapy within 4 weeks prior to enrollment.
12. Clinically significant infections (eg, systemic fungal infections) requiring systemic treatment.
13. Known immune disorder (including myeloma and lymphoma).
14. Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders.
15. An absolute neutrophil count <1000 cells/mm^3.
16. Markers of hepatic inflammation or cirrhosis as evidenced by 1 or more of the following:
   a. Platelet count of <150,000/µL.
   b. Serum albumin level is below the central laboratory's lower limit of normal and FibroSURE is ≥0.48 (ie, Metavir staging of F2 or greater). Of note, in subjects with a known history of Gilbert's syndrome, a Fibrotest cannot be used for fibrosis testing.

c. Total bilirubin >1.5 × ULN and direct bilirubin ≥0.5 mg/dL.
d. ALT or AST >1.0 × ULN.
e. Alkaline phosphatase >2.0 × ULN.
f. History of liver biopsy indicating moderate or severe fibrosis (Metavir staging of F2 or greater).
g. History of ascites, varices, variceal hemorrhage, or hepatic encephalopathy.
h. Any findings on screening ultrasound that would preclude the safe use of AAV gene therapy.

17. Prothrombin time (PT) international normalized ratio (INR) ≥1.4.
18. Serum creatinine >1.5 mg/dL.
19. Urine protein >30 mg/dL OR >0.5 g/day.
20. Body mass index >38.
21. Orthopedic surgery or other major surgery planned within 6 months after enrollment.
22. Acute or chronic disease that, in the opinion of the investigator, would adversely affect subject safety or compliance or interpretation of study results.
23. Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0.
24. Received an investigational intervention or participated in another clinical trial within 4 weeks prior to enrollment or within 5 half-lives of the investigational drug administration, whichever is longer.
25. 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).
26. 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.
27. Subject is a family member or employee of the investigator.

STATISTICAL ANALYSIS

Sample Size Calculation

The sample size is expected to range from 2 to 12 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.

Planned Statistical Analysis

In general, descriptive summaries will be presented for the primary, secondary, and tertiary outcome measures. 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. The primary outcome of the incidence of BAX 888-related AEs will be analyzed as a categorical variable. An exploratory, descriptive analysis will also be performed for events categorized as development of inhibitory antibodies to FVIII and total binding antibodies to FVIII, severe allergic reactions, and thrombosis-associated events. More detailed information about summarization of data, graphical representations, and analysis conventions will be provided in the Statistical Analysis Plan.

4. TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
AASLD	American Association for the Study of Liver Diseases
AAV	adeno-associated virus
ABR	annualized bleed rate
AE	adverse event
ALT	alanine aminotransferase
ANA	antinuclear antibody
AMA	antimitochondrial antibody
AST	aspartate aminotransferase
BDD	B-domain deleted
BU	Bethesda units
BUN	blood urea nitrogen
CK	creatine kinase
CMI	cell-mediated immune
CNS	central nervous system
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic lymphocyte
DMC	Data Monitoring Committee
EASL	European Association for the Study of the Liver
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EQ-5D	EuroQol-5D
FIX	factor IX
FVIII	factor VIII
GCP	Good Clinical Practice
GGT	gamma-glutamyl transpeptidase
Haemo-QoL-A	Hemophilia-Specific Quality of Life Index
HaemoPREF	Patients' Preference for Hemophilia Treatment Questionnaire
HAV	Hepatitis A virus

Abbreviation	Definition
HBc	hepatitis B core
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HIV	human immunodeficiency virus
HJHS	Hemophilia Joint Health Score
HLA	human leukocyte antigen
HRQoL	health-related quality of life
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IFN-γ	interferon-γ
IgG/IgM	immunoglobulin G/immunoglobulin M
IP	investigational product
IRB	Investigational Review Board
IV	intravenous
ITR	inverted terminal repeats
LDH	lactate dehydrogenase
LC 1	liver cytosol type 1
LFT	liver function test
LKM1	liver-kidney microsomal antibody type 1
NAbs	Neutralizing antibodies
NMC	non-medical complaint
PAMPs	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
polyA	short polyadenylation sequence
PRO	patient reported outcome
PT	prothrombin time
rAAV	recombinant adeno-associated virus
RBC	red blood cell
RNA	ribonucleic acid
RSI	Reference Safety Information

Abbreviation	Definition
SAE	serious adverse event
SAER	serious adverse event report
SAP	statistical analysis plan
SD	standard deviation
SF-36v2	36-item Short Form survey version 2
SIC	subject identification code
SMA	smooth muscle antibody
SUSAR	suspected unexpected serious adverse reaction
TTR	transthyretin
ULN	upper limit of normal
UPR	unfolded protein response
VERITAS-Pro	Validated Hemophilia Regimen Treatment Adherence Scale-Prophylaxis
vWF	Von Willebrand factor
WT	wild-type

5. BACKGROUND INFORMATION

5.1 Description of Investigational Product

The investigational product (IP), BAX 888, is a gene therapy product engineered to induce the production of human factor VIII (FVIII) protein in the liver cells of individuals with severe congenital FVIII deficiency. The BAX 888 drug product is based on an adeno-associated virus, serotype 8 (AAV8) vector expressing a functional human B-domain deleted, codon-optimized FVIII protein. 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 DNA sequence that is delivered in the BAX 888 vector is the FVIII gene along with DNA elements required for expression of the potentially therapeutic *F8* gene. The rationale for the choice of the serotype of 8 for the BAX 888 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 (eg, in antigen presenting cells). In addition, BAX 888 incorporates a hepatocyte specific promoter to further restrict FVIII expression to the target liver tissue.

BAX 888 is intended to be given as a single, one-time intravenous (IV) infusion by a health care provider, at a qualified Center. If successful transduction of hepatocytes is achieved, long-term hepatic expression and secretion of endogenous FVIII into the blood stream may support hemostasis and in turn eliminate or reduce the prophylactic and/or on-demand use of exogenous FVIII concentrate therapy in hemophilia A patients. For the initial Phase 1/2 clinical study, BAX 888 will be supplied as a 5 mL or 10 mL frozen, sterile, non-pyrogenic solution intended for IV infusion, and dosing will be based on the subject's body weight. The current safety study will include investigation of the proposed doses of 2.0×10^{12} cp/kg (lowest BAX 888 dose predicted to provide FVIII expression), 6.0×10^{12} cp/kg and a further dose of 1.2×10^{13} cp/kg (see proposed dose escalation scheme in Figure 3). 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 5.4 for further information on BAX 888 and its usage in this study. A detailed description of BAX 888 is also provided in the BAX 888 Investigator's Brochure (IB).

The study design is in compliance with the EMA/CHMP/BPWP/144533/2009 guidelines for the study of FVIII in hemophilia A (Committee for Medicinal Products for Human Use, 2009).

5.2 Clinical Condition/Indication

Hemophilia A is a rare congenital disease characterized by reduced or absent levels of the coagulation FVIII. It occurs in approximately 1 in 5,000 live male births. FVIII is encoded by *F8*, a large gene (186 kb) that is found in the most distal band (Xq28) of the long arm of the X chromosome. Thus, females are carriers of the disease, which almost exclusively affects males through inheritance from the maternal side. Mutations of the FVIII gene result in a congenital deficiency or defect in FVIII, a crucial factor in blood coagulation. In the absence of functional FVIII, the coagulation cascade is severely impaired resulting in a bleeding disorder, the severity of which is dependent on the residual endogenous levels of FVIII.

Severity of hemophilia is typically categorized as follows:

- Severe hemophilia: FVIII activity levels <1% of normal FVIII activity.
- Moderate hemophilia: FVIII activity levels ≥1 to 5% of normal FVIII activity.
- Mild hemophilia: FVIII activity levels >5 to <40% of normal FVIII activity.

Hemophilia A is characterized by easy bruising in early childhood, spontaneous bleeding (particularly into joints, musculoskeletal tissues, and other soft tissues causing significant pain, and permanent damage if left untreated), and excessive bleeding following trauma or surgery.

Current standard of care management of hemophilia A is to treat mainly by replacement of FVIII using prophylactic or on-demand regimens to control bleeding episodes, and ultimately to prevent joint damage and death in severe cases. For long-term prophylaxis against bleeding in patients with severe hemophilia A, the usual replacement doses are 30 to 40 IU of FVIII per kg body weight at intervals of 2 to 3 days. In patients under the age of 6, doses of 20 to 50 IU of FVIII per kg body weight 3 to 4 times weekly are recommended.

In comparison to the current treatment modalities, gene transfer in hemophilia A holds substantial promise in that it is considered to be a one-time intervention that is potentially curative. This intervention may lead to complete avoidance of the need for IV infusions and their side effects and, ultimately, would be life-changing for patients affected with this condition.

5.3 Population to be Studied

The target population for this Phase 1/2 first-in-human study is male subjects, aged 18 to 75 years inclusive, with severe hemophilia A, as evidenced by a FVIII:C level <1% following ≥5 days without FVIII treatment, and documented evidence of ≥3 hemorrhages over the previous 12 months requiring treatment with exogenous FVIII or use of FVIII prophylaxis due to a history of frequent bleeding episodes. All subjects will have previously been treated with exogenously administered FVIII concentrates or cryoprecipitate for >150 documented exposure days.

Subjects with a detectable FVIII inhibitor with a titer ≥0.6 Bethesda units (BU) at the time of screening or well documented personal laboratory evidence of a FVIII inhibitor with a titer ≥0.6 BU will not be eligible. Similarly, subjects with an inherited or acquired hemostatic defect other than hemophilia A and subjects with chronic hepatic dysfunction or severe renal impairment will not be eligible to participate. See Section 8.2.

5.4 Findings from Nonclinical and Clinical Studies

5.4.1 Nonclinical Studies

Nonclinical dose response and toxicology studies have been completed. Data from these and additional nonclinical studies can be found in the BAX 888 IB.

5.4.2 Clinical Studies

No prior studies of BAX 888 in humans have been performed.

5.5 Evaluation of the Unmet Medical Need in the Treatment of Severe Hemophilia A

Current standard of care therapy for severe hemophilia A consisting of continuous repeated prophylactic infusions of FVIII concentrates is effective in controlling most hemorrhages and slowing or preventing the development of hemophilic arthropathy and other chronic complications of bleeding. Major limitations to continuous prophylactic FVIII therapy include the painful and time-consuming nature of repeated IV therapy and the continuous need for access to clotting factor concentrate and infusion supplies, which are associated with poor adherence. A recent analysis of 4899 adult men with severe hemophilia in the United States multidisciplinary Hemophilia Treatment Center Network demonstrated that regular prophylactic clotting factor infusion was performed by less than 30% of participants (including less than 50% of men with severe hemophilia less than 30 years of age) (Mazepa et al., 2016). Despite relatively good access to clotting factor concentrates in the United States and other resource-rich countries (~75% of men across all age groups in this analysis practiced home infusion of factor concentrates) 39% reported having very frequent joint bleeding (≥5 joint bleeds per six month period) and 36% reported limitations of school/work and activities of daily living as a result of joint bleeding (Mazepa et al., 2016).

5.5.1 Evaluation of Anticipated Benefits and Risks of AAV-FVIII Gene Therapy

Rigorous efforts to decrease the burden and improve the care of the disease by prolonging the circulating half-life of infused FVIII concentrate have resulted in only modest improvements (<1.5 times prolongation of FVIII half-life). Since even a modest increase in the level of missing FVIII can ameliorate the severe bleeding phenotype, it is anticipated that the successful development of a hemophilia A gene therapy product would lead to continuous endogenous expression of FVIII and would address many of the current treatment limitations by achieving greater than 1% of circulating FVIII activity at all times.

Such an endpoint, if achievable, could convert the severe bleeding phenotype to that of moderate or mild hemophilia (which is the goal of FVIII prophylaxis).

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 IP BAX 888 has not been tested in humans previously. The primary objective of the current study is to evaluate safety and to determine a dose of BAX 888 that may lead to levels of FVIII expression that have been associated with freedom from spontaneous joint hemorrhage. As with all Phase 1 trials, there is not an expectation that study subjects will receive benefit, although participation is expected to provide benefit in further directing the development of BAX 888 hemophilia gene therapy. BAX 888 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 FVIII expression; however, there is no prior experience with BAX 888 in humans. For this same reason, if a single injection of BAX 888 directs FVIII expression in humans, the anticipated length of persistence of expression following a single injection of BAX 888 in humans is currently not known. 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 (Nienhuis et al., 2016).

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 adverse events (AE).

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). Nevertheless, at doses of rAAV gene therapy vectors used clinically, it is anticipated that all subjects will develop neutralizing antibodies (NAbs) against the capsid serotype. Although development of AAV-specific NAbs 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 NAbs 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 BAX 888 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 alanine aminotransferase – ALT) has been documented in some subjects on multiple hemophilia gene therapy and other AAV liver-directed gene therapy trials (Manno et al., 2006; Nathwani et al., 2014)[vii]. 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; Nathwani et al., 2014);[vii] 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.

[vii] NCT00076557 (https://clinicaltrials.gov/ct2/show/NCT00076557);
NCT00979238 (https://clinicaltrials.gov/ct2/show/NCT00979238);
NCT01620801 (https://clinicaltrials.gov/ct2/show/NCT01620801);
NCT01687608 (https://clinicaltrials.gov/ct2/show/NCT01687608);
NCT02484092 (https://clinicaltrials.gov/ct2/show/NCT02484092);
NCT02971969 (https://clinicaltrials.gov/ct2/show/NCT02971969)

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.

Preliminary results from an ongoing clinical study using the AAV5 vector to deliver the B-domain deleted (BDD) FVIII gene were recently published (Rangarajan et al., 2017). Six of seven subjects treated with high doses of rAAV5 BDD FVIII were reported to be expressing FVIII at normal or supernormal levels with at least 34 weeks of follow-up, with a seventh subject expressing FVIII at 15%. Although chronic expression of FVIII at >150% activity carries an increased relative risk of thrombosis, no thromboses have been reported in this or any other trial of AAV-directed gene therapy for hemophilia. Following elevation of ALT in the first high dose subject in the hemophilia A trial, all subsequent subjects were treated with prophylactic corticosteroids. The utility of prophylactic corticosteroids was not demonstrated in this trial, however, as all subjects developed mild transaminitis in the 1-2X normal range (ie, in the same range as the high dose individual who did not receive prophylactic corticosteroids, but instead received corticosteroid dosing only after developing transaminase elevation). Following prolonged taper of corticosteroids, the ALT levels of the seven subjects are reportedly at or near normal levels. The sponsors of this single study of AAV gene therapy for hemophilia A have concluded that prophylactic corticosteroids will not be used with future dosing.

Neutralizing antibodies (“inhibitors”) to FIX or FVIII have not developed in any AAV-directed trial of gene therapy for hemophilia. The specific concern that expression of FVIII 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, 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).

5.5.1.1 Prednisolone

Prednisolone is a highly potent glucocorticoid steroid having anti-inflammatory and immunosuppressive activity, as well as anti-proliferative, vasoactive, hormonal and metabolic effects qualitatively similar to those of hydrocortisone (Czaja and Manns, 2010; Liu et al., 2013). The anti-inflammatory and immunosuppressive properties of glucocorticosteroids result from upregulating the transcription of anti-inflammatory genes or by downregulating the transcription of inflammatory genes, leading to changes in downstream production of cytokine/chemokine proteins that initiate or maintain inflammation. Prednisolone, which is the active metabolite of prednisone, modifies the body's immune responses to diverse stimuli including autoimmune processes. Taking this into consideration, the American Association for the Study of Liver Disease (AASLD) recommendations for the treatment of autoimmune hepatitis, for example, specify a starting dose and tapering approach using the corticosteroid prednisone as a single agent (Manns et al., 2010; Soloway et al., 1972), while the European Association for the Study of the Liver (EASL) guidelines allow initiation of therapy with either prednisone or prednisolone (given at identical total dose) (European Association for the Study of the Liver, 2015).

Prednisolone toxicity is related to both the dose and cumulative duration of its use. For most prednisolone-related AEs, a precise threshold dose or duration of treatment is not established. Despite the beneficial effects of glucocorticoids, and the very common clinical use of prednisolone in a variety of disorders, the use of these agents (in particular long-term systemic use) is associated with a great variety of well-recognized adverse events, including: low bone mineral density; osteonecrosis; adrenal suppression; hyperglycemia and diabetes; cardiovascular disease and lipid dysregulation; sleep and psychiatric disturbances; and immune suppression (Liu et al., 2013).

NOTE: In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (eg, USA), prednisone will be utilized. Prednisone is converted to the active metabolite prednisolone in the liver and is recommended by the AASLD and the EASL for the treatment of autoimmune hepatitis.

5.5.1.2 Evaluation of Anticipated Benefits and Risks of Co-administration of Prednisolone and AAV

The BAX 888 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.

The protocol includes a prophylactic course of corticosteroids. As seen in prior AAV hemophilia gene therapy trials, subjects may demonstrate clotting factor expression followed by an apparent cytotoxic lymphocyte (CTL) response against transduced hepatocytes. The rationale to use corticosteroids in an attempt to prevent the course of the apparent 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 NAb. The rAAV-associated liver inflammation has in most individuals resolved rapidly (ie, within 2 weeks) (Nathwani et al., 2014) with the institution of high dose corticosteroid therapy, followed by taper over 8-12 weeks. What has varied between individuals enrolled in three different AAV hemophilia B gene therapy trials is that the degree to which the supportive institution of corticosteroids has been associated with sustained clotting factor expression has varied from 0% to 100%.

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 AASLD and the EASL for the treatment of autoimmune hepatitis (Nathwani et al., 2014; Manns et al., 2010; 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 to 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 (Liu et al., 2013; Ahmet et al., 2011). Subjects will be monitored for the development of Cushingoid features, as it is common within 2 months of start of therapy (Fardet et al., 2007). Hyperglycemia may occur and glucose will be assayed regularly in all study subjects. Glucocorticoids used for even short periods of time may lead to psychiatric and cognitive disturbances including memory impairment, irritability, mood lability and sleep disturbance.

The BAX 888 protocol specifies for subjects who are at increased risk from corticosteroid therapy, including individuals with osteoporosis and vertebral fractures, hard to control diabetes and hard to control hypertension, emotional instability, and obesity combination therapy with azathioprine/prednisone per AASLD guidelines which allows lower cumulative glucocorticoid dose and risk.

5.6 Compliance Statement

This study will be conducted in accordance with this protocol, the International Council for Harmonisation Guideline for Good Clinical Practice E6 (ICH GCP, April 1996), Title 21 of the US Code of Federal Regulations, the EU Directives 2001/20/EC and 2005/28/EC, and applicable national and local regulatory requirements.

This study will be conducted in a manner that assures that the rights, safety and well-being of the participating study subjects are protected consistent with the ethical principles that have their origin in the Declaration of Helsinki.

6. STUDY PURPOSE AND OBJECTIVES

6.1 Study Purpose

The purpose of the study is to evaluate the safety and determine the dose of BAX 888 required to achieve FVIII activity levels ≥20% of normal in severe hemophilia A subjects.

6.2 Primary Objective

The primary objective of the study is to evaluate the safety of a single IV infusion of BAX 888 in up to 3 dose cohorts.

6.3 Secondary Objectives

1. To evaluate plasma FVIII activity levels pre- and post-BAX 888 infusion and investigate the relationship between FVIII activity levels and BAX 888 dose.
2. To determine the BAX 888 dose needed to achieve FVIII activity level of ≥20% of normal in ≥60% of the subjects.
3. To collect bleeding rate and consumption of exogenous FVIII after gene transfer.
4. To assess humoral and cellular immune responses to FVIII and the AAV8 viral capsid.
5. To determine the duration of BAX 888 genome presence in blood, saliva, semen, urine and stool.

7. STUDY DESIGN

7.1 Brief Summary

This is a global, Phase 1/2 multicenter, open-label, safety, and dose escalation study of BAX 888 in adult subjects with severe hemophilia A. Up to 12 subjects may be administered BAX 888 in up to 3 dose cohorts.

Before any study-related procedures are followed, subjects will provide signed informed consent. During the course of the study, FVIII activity levels will be assessed by a central laboratory (both the one-stage and chromogenic assays) and by the local laboratory (either one-stage or chromogenic assay) as specified in Table 3. However, only central laboratory FVIII activity levels derived from the one-stage clotting assay will be utilized for the primary analysis. FVIII activity levels obtained from the central laboratory chromogenic assay will be utilized to support the one-stage assay results. BAX 888 will be administered as a single peripheral IV infusion. On the day of dosing with BAX 888 (Day 0), subjects will be admitted to the infusion center and will be observed for at least 8 hours following dosing. If the infusion center is not the subject's Hemophilia Treatment Center/local investigator's institution, a second separate consent will be obtained from the subject for the Day 0 visit and for all procedures surrounding the infusion period. Subjects will be monitored for the first 8 hours and may remain in the infusion center for 24 hours following the BAX 888 infusion at the discretion of the investigator or return to the center for the 24-hour post-infusion follow-up. All subsequent follow-up visits will take place at the subject's own Hemophilia Treatment Center/local investigator's institution at: Week 1 and Week 2 (once weekly), Weeks 3 to 14 (twice weekly, with separate clinic visit and laboratory visit each week), Week 15-18 (once weekly), and then Months 5, 6, 9, 12, 16, 20, 24, 28, 32, 36, 48, and 60.

Safety, efficacy, and health-related quality of life (HRQoL) data will be collected throughout the 5-year post-gene transfer period.

7.2 Overall Study Design

This is an open-label, Phase 1/2 study designed to evaluate the safety, efficacy, and pharmacodynamics of up to 3 doses of BAX 888. The overall study design is illustrated in Figure 2.

The target population is up to 12 evaluable adult male subjects with severe hemophilia A (FVIII:C level <1%). 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.

Subjects will be assigned to 1 of 3 dose cohorts (Figure 3), with a minimum of 24 hours between dosing of each subject. Initially, 2 subjects will be dosed in a cohort, with up to a total of 5 subjects if the cohort is expanded. Safety and FVIII activity level data from subjects dosed with BAX 888 will be utilized to make decisions on dose escalation and cohort expansion. If any subject in a given cohort achieves >50% FVIII activity levels at Week 14, the Data Monitoring Committee (DMC) will provide a recommendation on further dose escalations after review of all safety and FVIII activity level data. If any subject in any cohort achieves ≥150% FVIII activity levels at any time while on the study, further dosing will be paused until DMC review. Only central laboratory FVIII activity levels will be utilized for dose escalations. DMC recommendations will be based on, but not limited to, the following considerations: (1) mean, median, and range of FVIII activity levels (central lab, one-stage clotting assay) observed within the dosing cohort, (2) variability in response between subjects, (3) liver function tests, and (4) other product-related adverse events (AEs).

Cohort 1 (2.0×10^{12} cp/kg):

After dosing the first 2 subjects in Cohort 1:

- If Week 4 FVIII activity levels of both subjects are <2%, then dose escalation to Cohort 2 will be triggered with no further dosing in Cohort 1. Alternatively, if FVIII activity levels (≥2%) are observed in at least 1 subject, the decision to escalate dose or expand the cohort with dosing of additional subjects will be based on all available data through Week 14.
- If sustained Week 14 FVIII activity levels of ≥30%[viii] are not achieved in both subjects, then escalation to Cohort 2 will be triggered after DMC review of all safety and FVIII activity level data inclusive of Week 14.
- If sustained Week 14 FVIII activity levels of ≥30%[viii] are achieved in at least 1 of the 2 subjects, then expansion of Cohort 1 will be initiated with dosing of 3 additional subjects.

For an expanded Cohort 1 with 5 subjects dosed with BAX 888:

- If sustained Week 14 FVIII activity levels of ≥30%[viii] are not achieved in at least 3 of the 5 subjects, then escalation to Cohort 2 will be triggered after DMC review of all available safety and FVIII activity level data inclusive of Week 14.
- If sustained Week 14 FVIII activity levels of ≥30%[viii] are achieved in at least 3 of the 5 subjects, one of the following would be pursued based on safety, FVIII activity levels, and the variability in response that is observed among the 5 dosed subjects:

[viii] Sustained Week 14 FVIII activity level ≥30% is defined as 3 or more FVIII activity level measurements ≥30% between Week 12 and Week 18. If applicable per protocol, a DMC meeting will be held when either 3 or more FVIII activity level measurements ≥30% between Weeks 12 and Week 18 are obtained or after Week 18, whichever comes first.

1. Completion of study enrollment with no further dosing since the secondary objective to determine a BAX 888 dose needed to attain FVIII activity levels ≥20% in at least 60% of the subjects would have been achieved.
2. Expansion of Cohort 1 to include dosing of additional subjects at the same dose level.
3. Escalation to a higher dose (eg, subsequent patients will be enrolled in Cohort 2).

Cohort 2 (6.0×10^{12} cp/kg):

After dose escalation and administration of BAX 888 to the first 2 subjects in Cohort 2:

- If sustained Week 14 FVIII activity levels of ≥30%[viii] are not achieved in both subjects, then expansion of Cohort 2 or escalation to Cohort 3 will be triggered after DMC review of all available safety and FVIII activity level data inclusive of Week 14.
- If sustained Week 14 FVIII activity levels of ≥30%[viii] are achieved in at least 1 of the 2 subjects, then, after review of all the available safety and FVIII activity level data, the DMC will make a recommendation to continue with expansion of Cohort 2 up to 5 subjects, or escalate dosing to Cohort 3 (1.2×10^{13} cp/kg).

<u>For an expanded Cohort 2 with up to 5 subjects dosed with BAX 888:</u>

- If sustained Week 14 FVIII activity levels of ≥30%[v] are not achieved in at least 3 subjects, then one of the following would be pursued after DMC review of all available safety and FVIII activity level data inclusive of Week 14:
  - Escalation to Cohort 3 (1.2×10^{13} cp/kg)
  - Completion of study enrollment with no further dosing
- If sustained Week 14 FVIII activity levels of ≥30%[v] are achieved in at least 1 of the subjects, one of the following would be pursued after DMC review and based on the safety, FVIII activity levels, and the variability in response, and other clinical data that is observed among all the dosed subjects in the study:
  - Continue to expand Cohort 2 up to 5 subjects
  - Escalation to Cohort 3 (1.2×10^{13} cp/kg)
  - Completion of study enrollment with no further dosing

- Preliminary evaluation following 2 subjects dosed with 2.0×10^{12} cp/kg (lowest dose level) showed minimal impact on FVIII activity. Preliminary evaluation following 2 subjects dosed 6.0×10^{12} cp/kg (middle dose level; 3-fold higher than lowest dose level) suggests that the peak FVIII activity was as expected but increased more than proportional to the increase in dose. A mouse model suggested a potential non-linear dose-FVIII activity relationship (doubling of the dose might produce more than a doubling of the FVIII activity). To ensure informed dose escalation in subjects with hemophilia A, instead of a 3-fold increase in dose, the next proposed dose is 1.2×10^{13} cp/kg which is 2-fold the middle dose level originally proposed.

Cohort 3 (1.2 x 10^{13} cp/kg):

After dose escalation and administration of BAX 888 to the first 2 subjects in Cohort 3:

- If sustained Week 14 FVIII activity levels of ≥30%[viii] are not achieved in in both subjects, then dosing of additional subjects will be paused until further review of all available data.
- If sustained Week 14 FVIII levels of ≥30%[viii] are achieved in at least 1 of the 2 subjects, then expansion of Cohort 3 will be initiated with dosing of up to 3 additional subjects. Study could be completed with no further dosing.

Immunosuppression

Vector-derived factor expression followed by a decrease in factor levels has been observed in previous hemophilia gene therapy trials and has been attributed to an immune response directed against the successfully transduced cells. To address this issue and to ensure factor activity is achieved and sustained, BAX 888 has been engineered to have a reduced prospect of eliciting an immune response.

Nevertheless, a prophylactic course of glucocorticosteroid treatment with prednisolone as shown in Table 1 will be employed starting at Day 8 post-BAX 888 infusion. The following dosing schedule will be followed as a guideline. The corticosteroid regimen may be adjusted at the discretion of the Investigator in consultation with the Sponsor Medical Monitor depending on the subject's tolerance of the regimen and the observed hepatic transaminase response. Subjects receiving corticosteroids may require unscheduled additional visits to evaluate potential side effects of corticosteroid therapy and for corticosteroid dose adjustments. FVIII activity levels and liver function tests (LFTs) will be measured at these unscheduled visits by local laboratories.

Table 1. Prednisolone Dosing Regimen

Prednisolone Dose (mg/day)	Duration
60	4 weeks
40	2 weeks
30	2 weeks
25	2 weeks
20	2 weeks
15	1 week
10	1 week
5	1 week

Determine ALT and FVIII activity level weekly or twice weekly (as per study protocol).

In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (eg, 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.

The purpose of the prophylactic treatment with the tapering course of prednisolone is to prevent clinical or subclinical hepatic inflammation and to support the potential for sustained transgenic factor VIII expression.

Assessment of possible reactions to corticosteroids will be performed by the Investigator and modification of treatment instituted as necessary after consultation with the Sponsor's Medical Monitor. In such instances, consideration should be given to combination therapy with azathioprine/corticosteroid per AASLD guidelines or other drug as per standard of practice, in consultation with a hepatologist, so that the care can be individualized to the subject's needs. Recurrent elevations in transaminases after Year 1 should be evaluated and treated at the discretion of the investigator.

Full details on the procedures to be performed at each study visit, including screening, can be found in Section 19.1 (Study Flow Chart and Dose Escalation), Section 19.2 (Schedule of Study Procedures and Assessments), and Section 19.3 (Clinical Laboratory Assessments).

The following sections summarize the procedures and assessments to be performed at each visit.

7.2.1 Screen 1

Prior to Screen 1 visit, all subjects will undergo a minimum wash-out period of 5 days following their last FVIII therapy (on-demand or prophylaxis), after which blood samples will be collected to determine eligibility at Screen 1 visit. Baseline FVIII activity levels will be based on the central laboratory one-stage clotting assay and determined at the Screen 1 visit.

The following procedures and assessments will be performed:

- Signed informed consent for Screen 1 visit.
- Eligibility criteria checked.
- 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 FVIII 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. Full medical history could be collected throughout the screening period and finalized at the Screen 2 visit.

- 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.
- Blood and urine collected for laboratory assessments:

Hematology, coagulation and clinical chemistry panels, hepatitis B surface antigen, hepatitis C antibody, hepatitis C virus (HCV) ribonucleic acid (RNA), markers of autoimmune-mediated hepatitis, fibrinogen activity, D-dimer, FVIII genotyping, FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, antibody to FVIII and FVIII transgene product, neutralizing and binding antibodies to AAV8 and AAV2, cell-mediated immune (CMI) response to AAV8 and FVIII transgene products, and FibroSURE^{TM}, and urine protein.

If the Screen 1 visit occurs >180 days (±7 days) prior to the Screen 2 visit due to the implementation of Protocol Amendment 7, then study assessments may need to be repeated at the discretion of the Investigator and Sponsor. The study assessments to be repeated will be determined on a case-by-case basis. Sponsor approval should be received before moving forward with Screen 2 visit. Additionally, Screen 1 visit assessments that need to be repeated for subjects screened >180 days (±7 days) can be performed in a combined Screen 1 and Screen 2 visit.

7.2.2 Screen 2

The following procedures and assessments will be performed:

- Signed informed consent for visits encompassing Screen 2 and all subsequent Study Visits.
- Eligibility criteria checked.
- Medical history (any additional information that was not captured at Screen 1 or occurred between Screen 1 and Screen 2)
- Targeted physical exam (includes weight and examination of the liver, skin, and other organ systems as driven by signs, symptoms, and complaints).
- Vital signs.
- 12-lead electrocardiogram (ECG).

  Blood collected for laboratory assessments:

  - Hematology, coagulation, and clinical chemistry panels, FVIII inhibitor, neutralizing and binding antibodies to AAV8 and AAV2, Von Willebrand Factor (vWF) antigen, human leucocyte antigen (HLA) (MHC haplotype), HIV serology, and polymerase chain reaction (PCR) of vector genomes. Local laboratory LFTs including ALT, AST, and gamma-glutamyl transpeptidase (GGT). Whole genome sequencing if subject provides consent,
  - Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes.

- Bleeding episodes.
- Concomitant medications (including FVIII usage).
- Distribute electronic subject diaries (e-diary).
- AEs.
- Patient-reported outcomes (PROs).
- Abdominal ultrasound to assess liver health (should be performed locally; may be scheduled on different day, as close to Screen 2 visit as possible)

If the Screen 2 visit occurs >70 days prior to the Day 0 visit due to the implementation of Protocol Amendment 7, then study assessments may need to be repeated at the discretion of the Investigator and Sponsor. The study assessments to be repeated will be determined on a case-by-case basis. Sponsor approval should be received before moving forward with Day 0 visit.

7.2.3 Day 0

The following procedures and assessments will be performed:

- Signed informed consent (if the infusion center is not the subject's Hemophilia Treatment Center/local investigator's institution).
- Medical history (any additional relevant information that was not captured during screening visits or occurred between Screen 2 and Day 0)
- 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).
- 12-lead ECGs (up to 3 hours pre-infusion and at 1 hour and 6 hours post-infusion).
- BAX 888 administration.
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels, serum cytokines (interleukin-6 and tumor necrosis factor-α).
  - FVIII activity level (central and local laboratories) and FVIII antigen (pre-infusion [up to 3 hours prior to infusion]).
  - Neutralizing antibody to AAV8 and AAV2 (up to 3 hours pre-infusion).
  - Collection of whole blood for transcriptome (up to 3 hours pre-infusion and 8 hours post-infusion) and plasma / serum for metabolomics if left over backup sample is available.
  - Blood collected for backup (8 hours post-infusion).

- Review e-diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.
- Hemophilia Joint Health Score.

7.2.4 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 collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels, serum cytokines, fibrinogen activity, D-dimer, FVIII activity level (central and local laboratories), and PCR of vector genomes.
  - Collection of whole blood for transcriptome and plasma / serum for metabolomics analysis
  - Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes.
- Review e-diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.

7.2.5 Weeks 1-2

The following procedures and assessments will be performed weekly:

- Medical history.
- 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, HCV RNA (Week 1 only), fibrinogen activity, D-dimer, FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, neutralizing antibody to AAV8 and AAV2 (Week 2 only), CMI response to AAV8 and FVIII transgene products, and PCR of vector genomes (until results are negative for 2 consecutive time points).
  - Collection of whole blood for transcriptome and plasma / serum for metabolomics analysis.
  - Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points).
- Review e-diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.

7.2.6 Weeks 3-14

The procedures and assessments listed below will be performed at the first visit of the week (Clinic Visit). The Laboratory Visits are to include the local laboratory Liver Function Test, FVIII activity level assessment, and backup sample collection ONLY.

- Medical history.
- 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 (Weeks 3, 5, 7, 9, 11, & 13), Liver Function Tests (central [Weeks 4, 6, 8, 10, 12, & 14], and local [Weeks 3-14]), HCV RNA (Week 6), fibrinogen activity, D-dimer, vWF antigen (Week 6), FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, antibody to FVIII and FVIII transgene product (Weeks 6 and 8 only), neutralizing antibody to AAV8 and AAV2 (Weeks 4, 8, and 12 only), CMI response to AAV8 and FVIII transgene products, and PCR of vector genomes (until results are negative for 2 consecutive time points).
  - Collection of whole blood for transcriptome and plasma / serum for metabolomics analysis.

- Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points).
- Review e-diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.

The following laboratory assessments will be performed:

- Liver function tests to include ALT, AST and GGT.
  - Central Laboratory LFTs @ Clinic Visits during Weeks 4, 6, 8, 10, 12, and 14
  - Local Laboratory LFTs @ every Clinic and Laboratory Visit during Weeks 3-14.
- FVIII activity level.
  - Central Laboratory (one-stage and chromogenic assays) @ every Clinic and Laboratory Visit between Weeks 3-14.
  - Local Laboratory (one-stage or chromogenic assay) @ every Clinic and Laboratory Visit between Weeks 3-14.
- Blood collected for backup at every Laboratory Visit between Weeks 3-14.

7.2.7 Week 15-18

The following procedures and assessments will be performed weekly (Clinic Visit) except for the central laboratory LFTs:

- Medical history.
- 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 and clinical chemistry panels (Weeks 15 and 17 only), LFTs (central [Weeks 16 & 18], and local [Weeks 15 to 18]), coagulation panel, FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, CMI response to AAV8 and FVIII transgene products, PCR of vector genomes (until results are negative for 2 consecutive time points).
- Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points).

- Review diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.

7.2.8 Months 5, 6, 9, 12

The following procedures and assessments will be performed:

- Medical history (including hospitalizations, new malignancy[ies], new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of hematologic disorder, or any other new chronic medical conditions).
- Targeted physical exam (examination of the liver and skin, and otherwise driven by signs, symptoms, and complaints), includes weight at Month 12 only.
- Vital signs.
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels, fibrinogen activity, D-dimer, vWF antigen (Month 6), FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, antibody to FVIII and FVIII transgene product (Months 6, 9 and 12 only), neutralizing and binding antibodies to AAV8 and AAV2, CMI response to AAV8 and FVIII transgene products (Months 5, 6, and 12 only), and PCR of vector genomes (until results are negative for 2 consecutive time points).
  - FibroSURE (Month 12 only).
  - Collection of whole blood for transcriptome and plasma / serum for metabolomics analysis.
  - Alpha fetoprotein (optional assessment at local laboratory at Month 12 only)
  - Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points).
- Review e-diaries.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.
- Hemophilia Joint Health Score (at Month 12 only).

- PROs (at Months 6 and 12 only for HRQoL).
- Abdominal ultrasound to assess liver health (at Month 12 only; should be performed locally; may be scheduled on different day, but as close to Month 12 visit as possible)

Unscheduled visits will be performed if a >50% decrease in FVIII activity level from that of the last visit is observed at any time after Week 18. FVIII activity levels and liver enzyme levels will be assessed at these visits. Patients receiving corticosteroids may require additional visits to evaluate potential side effects of corticosteroid therapy and for dose adjustments, FVIII activity and liver enzyme levels will be measured at these visits.

7.2.9 Years 2-5 (Months 16, 20, 24, 28, 32, 36, 48, 60)

The following procedures and assessments will be performed:

- Medical history (including hospitalizations, new malignancy[ies], new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of hematologic disorder, or any other new chronic medical conditions).
- Physical exam (Months 24, 36, 48, and 60 only).
- Weight (Months 24, 36, 48, and 60 only).
- Vital signs.
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels, fibrinogen activity, D-dimer, FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, antibody to FVIII and FVIII transgene product, and neutralizing and binding antibodies to AAV8 and AAV2.
  - Alpha fetoprotein (optional assessment at local laboratory at Months 24, 36, 48, and 60 only)
  - Blood collected for backup.
- Saliva, urine, stool, and semen collected for PCR of vector genomes (until results are negative for 2 consecutive time points).
- Review and collect (at Month 60) e-diary.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.
- Hemophilia Joint Health Score (Months 24, 36, 48, and 60 only).
- PROs (Months 24, 36, 48, and 60 only for HRQoL).

- Abdominal ultrasound to assess liver health (at Month 24, 36, 48, and 60 only; should be performed locally; may be scheduled on different day, but as close to annual visit as possible)

Unscheduled visits will be performed if a >50% decrease in FVIII activity level from that of the last visit is observed at any time after Week 18. FVIII activity levels and liver enzyme levels will be assessed at these visits by Central Lab.

7.2.10 Early Termination Visit

The following procedures and assessments will be performed:

- Medical history (including hospitalizations, new malignancy[ies], new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of hematologic disorder, or any other new chronic medical conditions).
- Physical exam.
- Weight.
- Vital signs.
- Blood collected for laboratory assessments:
  - Hematology, coagulation and clinical chemistry panels, HCV RNA, fibrinogen activity, D-dimer, FVIII activity level (central and local laboratories), FVIII antigen, FVIII inhibitor, antibody to FVIII and FVIII transgene product, neutralizing antibody to AAV8 and AAV2, and CMI response to AAV8 and FVIII transgene products.
  - Collection of whole blood for transcriptome and plasma / serum for metabolomics analysis.
  - Blood collected for backup.
- Collect and review e-diary.
- Concomitant medications (including FVIII usage).
- Bleeding episodes.
- AEs.
- Recent physical activity.
- Hemophilia Joint Health Score.
- PROs.

7.2.11 Further Management of Presumptive Vector-Related Hepatitis

Any subject who develops an ALT or AST level >3 × upper limit of normal (ULN) during the study will continue to be treated with prednisolone[ix] as outlined in the Prednisolone Tapering Regimen tables above in the Immunosuppression Section (Section 7.2). Additionally, the subject must have the test repeated and confirmed within 72 hours.

In addition, if an increase in transaminase ALT or AST of 3 × ULN is observed, the additional clinical and laboratory monitoring should be initiated by the investigator. To ensure subject safety and comply with regulatory guidance, the investigator is to consult with the Sponsor Medical Monitor regarding collection of specific recommended clinical information and follow-up laboratory tests. If the level remains >3 × ULN, a diagnostic workup of potential infectious and toxic/metabolic causes of hepatitis will be pursued, including consultation with a hepatologist. This workup of potential etiologies for hepatitis (that may be unrelated to the Investigational product) will include careful history to assess potential liver diseases resulting from toxic exposures including alcoholic liver disease and laboratory evaluation by Central lab for potential viral co-infection with HBV, HCV, hepatitis E virus, hepatitis A virus as well as for non-infectious etiologies for hepatitis including hemochromatosis, autoimmune hepatitis, Wilson's disease, and alpha-1 antitrypsin deficiency.

The laboratory workup to be obtained includes the following studies, which may be supplemented by additional studies as determined to be appropriate for the individual case by the consulting hepatologist:

Potential viral co-infection: anti-HCV antibody, HCV RNA PCR, Hepatitis B surface antigen (HBsAg), anti-Hepatitis B core (HBc) immunoglobulin M (IgM) Ab, anti-Hepatitis A virus (HAV) IgM, anti-Hepatitis E virus (HEV) IgM.

- Hemochromatosis: serum ferritin (elevated iron stores), transferrin saturation.
- Autoimmune hepatitis: total serum immunoglobulin G (IgG), antinuclear antibody (ANA), SMA (smooth muscle antibody), anti- liver/kidney microsomes type 1 (LKM1) antibodies, anti- liver cytosol type 1 (LC 1), AMA (anti-mitochondrial antibody).
- Alpha1-antitrypsin deficiency: serum alpha antitrypsin.
- Wilson's disease: serum and urine copper, serum ceruloplasmin.

[ix] In geographies where prednisolone cannot be sourced at the strengths required for the specified dosing regimen (eg, 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.

Severe autoimmune hepatitis is defined by the American Association for the Study of Liver Diseases (AASLD) as an ALT or AST of ≥10 × ULN OR ALT or AST ≥5 × ULN with serum total IgG ≥2 × ULN or bridging necrosis on biopsy. Severe disease requires urgent treatment with corticosteroids. The AASLD evidence-based guidelines state that autoimmune hepatitis patients who have no, or only mild symptoms or laboratory abnormalities have very good immediate survival without corticosteroid therapy.

Management of presumptive vector-related hepatitis will be in accordance with a modified (more conservative) version of the most recent published guidelines by the AASLD: "Diagnosis and Management of Autoimmune Hepatitis" and the EASL for the treatment of autoimmune hepatitis[x], (European Association for the Study of the Liver, 2015).

If no other cause of the elevated ALT is identified, consideration should be given to combination therapy with azathioprine/corticosteroid per AASLD guidelines or other drug regimens as per standard of practice, in consultation with a hepatologist.

7.3 Duration of Study Period(s) and Subject Participation

The overall duration of the study is 8 years from study initiation (ie, first subject enrolled) to study completion (ie, last subject last visit). The recruitment period is expected to be about 40 months.

The subject participation period is approximately 5.5 years including the screening period and the 5-year post gene transfer follow-up.

7.4 Outcome Measures

7.4.1 Primary Outcome Measure

The primary outcome measure is the incidence of BAX 888-related AEs (serious or non-serious) including development of FVIII inhibitory antibodies, clinically significant changes in standard laboratory parameters, physical exam, and vital signs that are reported as AEs.

7.4.2 Secondary Outcome Measures

7.4.2.1 Efficacy

1. Circulating plasma FVIII activity and antigen levels.
2. ABR in comparison to before gene transfer.
3. Consumption of exogenous FVIII in comparison to before gene transfer.

---

[x] https://www/aasld.org/sites/default/files/guideline_documents/autoimmunehepatitis2010.pdf: date last accessioned: 14/Dec/2015.

7.4.2.2 Safety

1. Development of inhibitory and total binding antibodies to FVIII.
2. Humoral and cell-mediated immune response to AAV8 and FVIII proteins.
3. Surveillance of AAV8 genome shedding in blood, saliva, semen, urine and stool.

7.4.3 Tertiary Outcome Measures

1. Hemophilia Joint Health Score at screening and Months 12, 24, 36, 48, and 60 post-gene transfer.
2. Changes in the following assessments:
   - HRQoL (post-gene transfer vs. baseline) measured by:
     - Generic: 36-item Short Form survey (SF-36v2), EuroQol-5D (EQ-5D).
     - Disease-specific: Hemophilia-Specific Quality of Life Index (Haemo-QoL-A).
3. Patient Experience (baseline):
   - Adherence: Validated Hemophilia Regimen Treatment Adherence Scale-Prophylaxis (VERITAS-Pro).
   - Disease-specific patient experience: Patients' preference for hemophilia treatment questionnaire (HaemoPREF).

7.5 Randomization and Blinding

This is a non-randomized, open-label, active treatment, dose escalation clinical study.

7.6 Study Stopping Rules

Each subject will receive only one dose of BAX 888 during the course of the study; hence stopping rules apply only during the screening. Subjects who have been dosed with BAX 888 will be followed to the end of the study as per protocol.

If any one of the following criteria are met, BAX 888 dosing will be paused and further enrollment will be halted until an independent DMC evaluates all available study data and makes a recommendation:

- More than 3 × ULN increase in ALT, AST, or both in any subject after BAX 888 administration that is not manageable by steroid rescue treatment implemented according to AASLD guidelines.
- A serious adverse event (SAE) that may or may not be potentially related to BAX 888 and which poses either an immediate risk to 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 the IP.

- The development, after having received BAX 888, of an inhibitor towards FVIII in any subject will lead to pausing of dosing and further investigations of the characteristics and potential contributing factors and causal relationships of the observed FVIII inhibitor.
- Death of a subject, after having received BAX 888, that is judged as definitely, probably, or possibly attributed to BAX 888. The study will be temporarily stopped in order to undergo review by the Investigational Review Board (IRB), DMC and the FDA.
- The occurrence of a malignancy at any point after gene transfer that is judged as probably or possibly related to BAX 888.

The study may be terminated, if one or more of the following criteria are met:

1. The sponsor decides to stop enrollment into the study based upon its assessment of safety.
2. The sponsor decides to stop enrollment into the study for administrative reasons.

7.7 Investigational Product(s)

7.7.1 Packaging, Labeling, and Storage

Product Name: BAX 888

Vector Name (Biologic Substance)

Serotype: AAV8

Gene Insert: *F8* Transgene

BAX 888 contains an AAV genome consisting of a modified (truncated) 320 bp transthyretin (pre-albumin, TTR) promoter/enhancer, followed by a 77 bp intron fragment from minute virus of mice-MVM, driving expression of a codon-optimized BDD *F8* cDNA. The codon optimization algorithm results in conservation of the wild type FVIII amino acid sequence without any mutation introduced. The B domain of the FVIII is deleted with the SQ linker at the site of the deletion; this SQ linker is the same sequence that is used in the commercially available recombinant FVIII proteins Refacto® and Xyntha®. The gene insert has a synthetic short polyadenylation sequence (polyA). The insert is bounded by the 145 bp AAV2 inverted terminal repeats (ITR).

Both terminal repeats have the wild type 145 nucleotide sequence, so as to direct replication and packaging of conventional single strand AAV DNA sequences. The biological function of the gene expression cassette has been tested by transfection, viral vector packaging, and *in vivo* intravenous injection into hemophilia A mice.

The AAV is a physically stable capsid of approximately 25 nm. The virus structural proteins are VP1, VP2, and VP3, and occur in a reproducible ratio of 1:1:10, respectively; these proteins are routinely assayed by SDS-PAGE with silver staining. Figure 1 shows the vector DNA structure.

Figure 1. Vector DNA Structure

BAX 888 is provided as a clear and sterile aqueous solution in sterilized, pyrogen-free and low-virus-binding SiO_2-coated glass vials as described on the label. The final product formulation consists of a solution of BAX 888 in 100 mM NaCl and 5% Trehalose. The product is filled at a target concentration of about 1 x 10^{13} cp/mL as described on the vial label; however, during the course of the study, a product with a higher concentration may become available. The information will be provided to the site to calculate the subject's dose as described in the Pharmacy Manual.

BAX 888 will be manufactured and packaged under current Good Manufacturing Practice conditions.

BAX 888 will be stored at -60°C or below, in a secured location.

Handling and disposal of BAX 888 will comply with standards for Biosafety Level 1 vectors and universal precautions.

For additional information please refer to the BAX 888 IB and/or other specific instructions provided by the Sponsor.

7.7.2 Administration

BAX 888 is to be administered as a single peripheral IV infusion. All ancillary supplies will be provided to the infusion sites for use in preparation and infusion of the BAX 888 product.

The BAX 888 Study Pharmacy Manual provides detailed step-by-step guidance on handling and administration of the study drug.

Briefly, a qualified pharmacist and/or designee will thaw BAX 888 and pool the calculated amount into appropriate syringes and dispense the IP to the investigator. The individual infusion volume will be based on the subject's assigned dose and body weight collected at the Screen 2 Visit.

Initially, approximately 10% of the BAX 888 infusion volume will be administered by manual push followed by an observation period of 30 minutes to monitor for infusion reactions. The remaining 90% of the BAX 888 product will then be administered as specified in the Pharmacy Manual.

7.7.2.1 Monitoring and Treatment of Allergic Reaction to BAX 888

All subjects will be monitored for allergic reactions following the initial infusion and the completion of the infusion of BAX 888.

Subjects who exhibit the following signs and symptoms will be treated accordingly (grading will be made in accordance with the Common Terminology Criteria for Adverse Events [CTCAE], Version 4.0, as described below) (Cancer Therapy Evaluation Program, 2009).

Grade 1 or 2 signs or symptoms consistent with allergic reaction:

Subjects should be reassessed at 5-minute intervals. Symptomatic treatment (antihistamine, nonsteroidal anti-inflammatory drug, acetaminophen and corticosteroid) is permitted by oral or parenteral route, but the symptoms must resolve within 30 minutes to be considered Grade 1 or 2. If the symptoms resolve within 30 minutes, an additional approximate 10% of the total dose of BAX 888 may be administered and an identical assessment performed over the subsequent 30 minutes. If there is no recurrence of symptoms, the remainder of the dose may be administered as specified in the BAX 888 Pharmacy Manual. If symptoms do not resolve within 30 minutes, the event will be considered Grade 3, and BAX 888 will be permanently discontinued. If there is a recurrence of symptoms, the infusion will be permanently discontinued. In all cases, the AE should be recorded and reported as per Section 11.1.

Grade 3 or higher signs or symptoms consistent with allergic reaction or anaphylaxis:

The infusion of BAX 888 must be permanently discontinued. The AE will be recorded and reported as per Section 11.1, and immediately reported to the Medical Monitor and DMC.

Additional assessment and treatment of the event should be at the investigator's discretion.

7.7.3 Description of the Investigational Product

See Section 7.7.1.

7.7.4 Investigational Product Accountability

The investigator or his/her designee/pharmacist will ensure that the IP(s) is stored as specified in the protocol and that the storage area is secured, with access limited to authorized study personnel.

The investigator or his/her designee/pharmacist will maintain records that the IP(s) was received, including the date received, drug identity code, date of manufacture or expiration date, amount received, and disposition. IP(s) must be dispensed only at the infusion center. Records will be maintained that includes the subject identification code (SIC), dispensation date, and amount dispensed. All remaining partially used and/or unused IP(s) will be returned to the sponsor or sponsor's representative after study completion/termination or destroyed with the permission of the sponsor in accordance with applicable state and federal laws, ICH-GCP guidelines, and study site procedures. If IP(s) is to be destroyed, the investigator or his/her designee/pharmacist will provide documentation in accordance with sponsor's specifications.

7.8 Source Data

Per ICH GCP, source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial that are necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies), which may be in paper and/or electronic format. Source data for this study comprise the following: hospital records, medical records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, outcomes reported by subjects, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical study.

No data will be entered directly onto the case report form (CRF).

For additional information on study documentation and eCRFs, see Section 16.2. The use of subject e-diaries is described in Section 9.5.

8. SUBJECT SELECTION, WITHDRAWAL, AND DISCONTINUATION

8.1 Inclusion Criteria

Subjects who meet **ALL** of the following criteria are eligible for this study:

1. Male, aged 18 to 75 years at the time of screening.
2. Established severe hemophilia A (FVIII:C <1%, measured following ≥5 days without FVIII treatment), **and/or** documented intron 1 inversion or intron 22 inversion mutation in the *F8* gene, consistent with severe hemophilia A, **AND** documented evidence of ≥3 hemorrhages over the previous 12 months requiring treatment with exogenous FVIII **or** use of FVIII prophylaxis because of history of frequent bleeding episodes.
3. History of >150 exposure days to exogenously administered FVIII concentrates or cryoprecipitate.
4. Sexually active men must agree to use barrier contraception (combination of a condom and spermicide) or limit sexual intercourse to post-menopausal, surgically sterilized, or contraception-practicing partners for a minimum of 6 months after administration of BAX 888, or until BAX 888 genomes are no longer detected in the semen, whichever is sooner.
5. Subject is willing and able to comply with the requirements of the protocol, including provision of semen samples, maintenance of a diary of bleeding episodes and FVIII protein use.
6. Signed informed consent.

8.2 Exclusion Criteria

Subjects who meet **ANY** of the following criteria are not eligible for this study:

1. Bleeding disorder(s) other than hemophilia A.
2. Personal laboratory evidence of having developed inhibitors to FVIII protein at any time (≥0.6 BU on any single test).
3. Documented prior allergic reaction to any FVIII 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 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, difficult to control hypertension, and difficult to control diabetes).

7. Evidence of markers of potential underlying risk for autoimmune mediated hepatic disease:
    a. Anti-smooth muscle antibody assay results ≥40 (Inova QUANTA Lite™ Actin IgG enzyme-linked immunosorbent assay [ELISA]); values of 31 to 39 will be flagged as possibly abnormal and the Investigator and Medical Monitor will evaluate the subject for eligibility.
    b. Elevated anti- LKM1 titers.
    c. Total IgG >1.5 × ULN.
    d. Antinuclear antibody titer >1:320; OR ANA titer >1:80 if demonstrated concurrently with ALT that is >ULN.
8. Active Hepatitis C: As indicated by detectable HCV RNA by PCR.
9. Hepatitis B: If surface antigen is positive.
10. Seropositive for Human Immunodeficiency Virus (HIV).
11. Receiving systemic antiviral and/or interferon therapy within 4 weeks prior to enrollment.
12. Clinically significant infections (eg, systemic fungal infections) requiring systemic treatment.
13. Known immune disorder (including myeloma and lymphoma).
14. Concurrent chemotherapy or biological therapy for treatment of neoplastic disease or other disorders.
15. An absolute neutrophil count <1000 cells/mm^3.
16. Markers of hepatic inflammation or cirrhosis as evidenced by 1 or more of the following:
    a. Platelet count of <150,000/µL.
    b. Serum albumin level is below the central laboratory's lower limit of normal and FibroSURE is ≥0.48. (ie, Metavir staging of F2 or greater). Of note, in subjects with a known history of Gilbert's syndrome, a Fibrotest cannot be used for fibrosis testing.
    c. Total bilirubin >1.5 × ULN and direct bilirubin ≥0.5 mg/dL.
    d. ALT or AST >1.0 × ULN.
    e. Alkaline phosphatase (AP) >2.0 × ULN.
    f. History of liver biopsy indicating moderate or severe fibrosis (Metavir staging of F2 or greater).
    g. History of ascites, varices, variceal hemorrhage, or hepatic encephalopathy.
    h. Any findings on screening ultrasound that would preclude the safe use of AAV gene therapy.
17. Prothrombin time (PT) international normalized ratio ≥1.4.
18. Serum creatinine >1.5 mg/dL.
19. Urine protein >30 mg/dL OR >0.5 g/day.

20. Body mass index >38.
21. Major surgery or an orthopedic surgical procedure planned within 6 months after enrollment.
22. Acute or chronic disease that, in the opinion of the investigator, would adversely affect subject safety or compliance or interpretation of study results.
23. Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0.
24. Received an investigational intervention or participated in another clinical trial within 4 weeks prior to enrollment or within 5 half-lives of the investigational drug administration, whichever is longer.
25. 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).
26. 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.
27. Subject is a family member or employee of the investigator.

8.3 Withdrawal and Discontinuation

Any subject may voluntarily withdraw (ie, reduce the degree of participation in the study) consent for continued participation and data collection. The reason for withdrawal will be recorded on the End of Study eCRF. Assessments to be performed at the termination visit (including in cases of withdrawal or discontinuation) are described in Section 9.6 and Section 19.1.

Discontinuation (ie, complete withdrawal from study participation) may be due to dropout (ie, active discontinuation by subject) or loss to follow-up (ie, discontinuation by subject without notice or action). Additionally, the investigator and sponsor have the discretion to discontinue any subject from the study if, in their judgment, continued participation would pose an unacceptable risk for the subject.

Subjects also will be withdrawn during the screening period for the following reasons:

- The subject develops a confirmed FVIII inhibitory antibody (≥0.6 BU by Nijmegen modification of the Bethesda assay).
- The subject is non-compliant with study procedures, in the opinion of the investigator.
- The subject would require major emergency surgery within 6 months of dosing.

Subjects also may be discontinued from further study participation post-infusion for the following reason, although every effort to collect follow up safety data will be made:

- The subject is non-compliant with study procedures, in the opinion of the investigator.

Subjects who received BAX 888 will not be replaced.

All withdrawn or discontinued subjects need to complete the final study assessments to be performed at the termination visit as described in Section 19.1.

9. STUDY PROCEDURES

9.1 Informed Consent

Any subject who provides informed consent (ie, signs and dates the informed consent form [ICF] and assent form, if applicable) is considered a subject in the study.

9.2 Subject Identification Code

The following series of numbers will comprise the SIC: protocol identifier (eg, 201501) to be provided by the sponsor, 2- or 3-digit number study site number (eg, 02) to be provided by the sponsor, and 3- or 4-digit subject number (eg, 0003) reflecting the order of providing informed consent. For example, the third subject who signed an ICF at study site 02 will be identified as Subject 201501-020003. All study documents (eg, eCRFs, clinical documentation, sample containers, drug accountability logs, etc.) will be identified with the SIC. Additionally, a uniquely coded SIC(s) is permitted as long as it does not contain a combination of information that allows identification of a subject (eg, collection of a subject's initials and birth date would not be permitted), in compliance with laws governing data privacy.

9.3 Screening and Study Visits

In general, subjects will undergo at least 2 screening visits (1 and 2) prior to dosing with BAX 888 (refer also to Section 7.2. Initially, subjects will provide consent for procedures and assessments to be performed at the first screening visit. The rationale for the Screen 1 visit is that it is expected that approximately 30 to 35% of the adult hemophilia population will have circulating antibodies that recognize and may neutralize AAV8. Treatment with BAX 888 is expected to be sub-therapeutic or non-therapeutic in the presence of such evidence of adaptive immunity. Therefore, subjects may elect to perform Screen 1 initially and to only complete the full Screen 2 if eligible based upon the results of Screen 1. Subjects who meet all inclusion and exclusion criteria will then advance to Screen 2, which will take place approximately 3 weeks but ≤70 days prior to dosing with BAX 888. Subjects will provide consent for all procedures and assessments to be performed at the Screen 2 and all following Study Visits. A targeted physical exam will be conducted at the Screen 2 visit. The rationale for the Screen 2 visit is to ensure that the subject does not have covert liver inflammation that has developed since the time of Screen 1 and does not have unrecognized immune responses to AAV or FVIII that have developed since the time of Screen 1, because the new development of either of these findings might compromise the safety and efficacy of BAX 888 therapy. E-diaries to capture subject reported data including bleeding episodes and FVIII usage will also be distributed at the Screen 2 visit.

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, 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 (eg, delay in timely availability of laboratory results).

9.4 Medications and Non-Drug Therapies

The following medications and non-drug therapies are **not** permitted within 4 weeks prior to dosing and during the course of the study (unless stated otherwise):

- Medications:
  - 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.
  - Received an AAV vector previously or any other gene transfer agent in the previous 12 months prior to Study Day 0.
  - An investigational intervention within 4 weeks prior to dosing or within 5 half-lives of the investigational drug administration, whichever is longer.
- 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 following medications and non-drug therapies are permitted within 4 weeks before study entry and during the course of the study:

- 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.
  - FVIII products

- 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 (eg, physiotherapy) deemed necessary by the subject's physician to treat or prevent any medical condition.

9.4.1 Use of Exogenous FVIII Throughout the Study

Clinical bleeding episodes will be managed with infusion of exogenous FVIII. Usage of exogenous FVIII for the prevention (prophylactic treatment) or episodic management (on-demand FVIII 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 FVIII activity levels derived specifically from the BAX 888 transgene and not exogenous FVIII products, subjects will be asked to refrain from prophylactic FVIII usage between Weeks 3 to 18 post-BAX 888 administration and during the 3 weeks prior to the Month 9 Visit. During these periods which are critical for the evaluation of sustained FVIII 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 FVIII product (rather than an extended half-life FVIII concentrate).

All use of exogenous FVIII will be recorded by the subject in the e-diary (source document) and transcribed by the site to the eCRF.

It is recognized and expected that suspected or evident bleeding episodes will be treated with infused FVIII protein as per the standard of care. If possible, study visits should be scheduled at least 3 to 5 days after a FVIII dose to minimize the chance that any FVIII activity level measured at the study visit results from exogenously administered FVIII. However, with the exception of Screen 1, study visits should not be postponed if the study subject has infused FVIII (eg, for the treatment of bleeding) within 4 days of the scheduled study visit.

9.5 Subject Diary and Patient Reported Outcomes

9.5.1 Subject Diary

An e-diary will be provided to each subject at the Screen 2 visit to record the following information:

1. Details of bleeding episodes.

2. Details of exogenous FVIII consumption.
3. Concomitant medications and non-drug therapies.
4. Untoward events.
5. Patient reported outcomes (see Section 11.10).

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, eg, joint, soft tissue, muscle, body cavity, intracranial, other.
- Type of bleed, ie, spontaneous (definitely no injury/trauma), injury (definitely due to injury/trauma), or unknown.
- Severity of bleed:
  - Minor: Early hemarthrosis, mild muscle bleeding (see Section 19.4.2), 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 e-diary, which will be provided to the subject in electronic format at Screen 2 Visit and will remain with the subject for the duration of the study. The investigator will review the e-diary for completeness and request missing information periodically and in a timely manner. Untoward events recorded in the e-diary will be reported as AEs according to the investigator's discretion and clinical judgment.

Subject entries in the e-diary will serve as source records. During study participation the investigator has access to the database holding the subject e-diary data.

After study closure, the investigator will receive the e-diary records for their subjects, including audit trail records, in PDF format. The data will be transmitted to the eCRF by a validated transfer.

9.5.2 Patient Reported Outcomes

The PRO instruments to be measured in this study are described below. These measures will be administered in this study at the time points noted in Table 2.

1. **Short Form-36:** The SF-36v2 is a self-administered, validated questionnaire designed to measure generic HRQoL. This 36-item questionnaire measures 8 domains, including: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. Two summary scores can be calculated, the Physical Component Score and the Mental Component Score; additionally, scores can be calculated for each of the 8 domains. Higher scores indicate better health status.
2. **EuroQol-5D:** The EQ-5D is a self-administered, standardized measure of health status that provides a generic measure of health for clinical and economic appraisal consisting of the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression each with 5 levels.
3. **Haemo-QoL-A:** The Haemo-QoL-A is a validated hemophilia A–specific instrument covering 6 areas (physical functioning, role functioning, worry, consequences of bleeding, positive affect, and treatment concerns) (Rentz et al., 2008).
4. **VERITAS-Pro:** VERITAS-Pro is a self/parent-reported questionnaire consisting of 24 questions on 6 (4-item) subscales (Time, Dose, Plan, Remember, Skip, Communicate) to assess adherence to prophylactic treatment. Lower scores reflect higher adherence (Duncan et al., 2010).
5. **HaemoPREF:** HaemoPREF is a 14-item questionnaire designed to measure ease of use and patients' preference for hemophilia treatment (Teal et al., 2014).
6. **Patient Activity Level:** Subjects will be asked to estimate their activity levels. This will consist of a few questions asking subjects to rate their current level of activity.

After study closure, the investigator will receive the PRO records for their subjects, including audit trail records, in PDF format. The data will be transmitted to the eCRF by a validated transfer.

9.6 Subject Completion/Discontinuation

A subject is considered to have completed the study when he/she ceases active participation in the study because the subject has, or is presumed to have, completed all study procedures according with the protocol (with or without protocol deviations).

Reasons for completion/discontinuation will be reported on the Completion/Discontinuation eCRF, including: completed, screen failure, AE (eg, death), discontinuation by subject (eg, lost to follow-up [defined as 3 documented unsuccessful attempts to contact the subject], dropout), physician decision (eg, progressive disease, non-compliance with IP/protocol violation[s], recovery), study terminated by sponsor, or other (reason to be specified by the investigator, eg, technical problems). Regardless of the reason, all data available for the subject up to the time of completion/discontinuation should be recorded on the appropriate eCRF.

Every effort will be made to have discontinued subjects complete the study completion/termination visit. If the completion/termination visit is done as an additional, unscheduled visit, the assessment results shall be recorded with the completion/termination visit. If a subject terminates participation in the study and does not return for the completion/termination visit, their last recorded assessments shall remain recorded with their last visit. The reason for discontinuation will be recorded, and the data collected up to the time of discontinuation will be used in the analysis and included in the clinical study report. If additional assessments are required, the assessments shall be recorded separately. Assessments to be performed at the termination visit (including in cases of withdraw or discontinuation) can be found in Table 2 and Table 3.

In the event of subject discontinuation due to an AE, clinical and/or laboratory investigations that are beyond the scope of the required study observations/assessments may be performed as part of the evaluation of the event. These investigations will take place under the direction of the investigator in consultation with the sponsor, and the details of the outcome may be reported to the appropriate regulatory authorities by the sponsor.

9.7 Procedures for Monitoring 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.

9.8 Communication with Sites

Procedures will be in place to ensure information is communicated in a timely fashion to all Investigators across all participating sites. Decisions pertaining to dosing, DMC communications, and any relevant safety information will be communicated to the Investigators and the sites in the form of an email. An acknowledgement of receipt of the email from the Investigator or designee will be required. Failure to receive acknowledgment will result in redundant notification by overnight mail.

10. ASSESSMENT OF EFFICACY

10.1 Circulating Plasma FVIII Activity and Antigen Levels

The following assessments will be made at the time points presented in Table 3 as part of the analysis and interpretation of efficacy outcome measures:

- FVIII genotyping.
- FVIII activity level (Central Laboratory: one-stage clotting and chromogenic assays; Local Laboratory: one-stage clotting or chromogenic assay).
- FVIII antigen.

10.2 Number of Bleeding Episodes Post-BAX 888 Infusion

The primary measure of hemostatic efficacy is the number of bleeding episodes post-BAX 888 administration. The number of bleeding episodes will be assessed based upon each individual bleeding episode, spontaneous or traumatic, recorded in the subject's e-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 (eg, pain consistent with a joint bleed [see Section 19.4.1]) or objective evidence of bleeding which may or may not require treatment with FVIII. Bleeding episodes occurring at the same anatomical location (eg, right knee) with the same etiology (eg, 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 (eg, knee and ankle bleeds following a fall) will be counted as a single bleeding episode.

Adverse events 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 (Section 11.1).

Hemophilia-related events meeting the criteria for seriousness will be reported as SAEs and described on the SAER form.

10.3 Consumption of Exogenous FVIII Compared to before Gene Transfer

Data pertaining to the consumption of exogenous FVIII during the 12-month period prior to BAX 888 infusion will be collected and recorded as part of the medical history. Post-gene transfer consumption of exogenous FVIII will be measured at the time points in Table 2.

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

11. ASSESSMENT OF SAFETY

11.1 Adverse Events

11.1.1 Definitions

An AE is defined as any untoward medical occurrence in a subject administered an IP that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom (eg, rash, pain, discomfort, fever, dizziness, etc.), disease (eg, peritonitis, bacteremia, etc.), or outcome of death temporally associated with the use of an IP, whether or not considered causally related to the IP.

11.1.1.1 Serious Adverse Event

A **serious** adverse event is defined as an untoward medical occurrence that at any dose meets one or more of the following criteria:

1. Outcome is fatal/results in death (including fetal death).
2. Is life-threatening – defined as an event in which the subject was, in the judgment of the investigator, at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe.
3. Requires inpatient hospitalization or results in prolongation of an existing hospitalization - inpatient hospitalization refers to any inpatient admission, regardless of length of stay.
4. Results in persistent or significant disability/incapacity (ie, a substantial disruption of a person's ability to conduct normal life functions).
5. Is a congenital anomaly/birth defect.
6. Is a medically important event – a medical event that may not be immediately life-threatening or result in death or require hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definitions above. Examples of such events are:
   - Intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependence or drug abuse.
   - Reviewed and confirmed seroconversion for, HAV, HBV, HCV, HEV, or parvovirus B19 (B19V).
   - Development of a confirmed inhibitor to FVIII with an inhibitor level ≥0.6 BU using the Nijmegen modification of the Bethesda assay.
   - Severe hypersensitivity/anaphylactic reactions to BAX 888.
   - 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.

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 BAX 888 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.

11.1.1.2 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any suspected adverse reaction to study treatment (ie, 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

Once determined to meet the criteria for a SUSAR, a SAE should be submitted to regulatory agencies expeditiously.

11.1.1.3 Non-Serious Adverse Event

A **non-serious** AE is an AE that does not meet the criteria of an SAE.

11.1.1.4 Unexpected Adverse Events

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 and/or prescribing information as occurring with a class of products or as anticipated from the pharmacological properties of the product, but are not specifically mentioned as occurring with the particular product under investigation. In addition, several human clinical trials using AAV2, AAV5 or AAV8 delivered via the systemic circulation have been associated with asymptomatic liver inflammation as evidenced by elevated liver transaminases (in particular, ALT elevations above the subject’s baseline) observed by laboratory evaluation. Liver enzyme elevation is a known complication for this drug class and is considered to be an expected complication of interest.

The expectedness of AEs will be determined by the sponsor using the IB and/or prescribing information 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 from the pharmacological properties of a product.

11.1.1.5 Preexisting Diseases

Preexisting diseases that are present before entry into the study are described in the medical history, and those that manifest with the same severity, frequency, or duration after IP exposure, will not be recorded as AEs. 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.

11.1.2 Assessment of Adverse Events

For the purposes of this study, the following non-serious events experienced after the first IP exposure are 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 FVIII.
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, eg, 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 (eg, a fall), the injury would not be reported as an AE, unless it resulted in a medical finding other than a bleeding episode (eg, abrasion of skin; fractured tibia). Therefore, any hemophilia-related event (eg, 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 (eg, a gastrointestinal hemorrhage requiring hospitalization) will be reported as SAEs and described on the SAER form.

All other AEs from the first IP exposure until study completion/discontinuation will be described on the AE eCRF using the medical diagnosis (preferred), or, if no diagnosis could be established at the time of reporting the AE, a symptom or sign, in standard medical terminology in order to avoid the use of vague, ambiguous, or colloquial expressions (see definition in Section 11.1). Each AE will be evaluated by the investigator for:

1. Seriousness as defined in Section 11.1.1.1.

2. Severity as defined in Section 11.1.2.1.
3. Causal relationship to IP exposure or study procedure as defined in Section 11.1.2.2.

For each AE, the outcome (ie, recovering/resolving, recovered/resolved, recovered/resolved with sequelae, not recovered/not resolved, fatal, unknown) and if applicable action taken (ie, dose increased, dose not changed, dose reduced, drug interrupted, drug withdrawn, not applicable, or unknown) will also be recorded on the AE eCRF. 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. If the severity rating for an ongoing AE changes before the event resolves, the original AE report will be revised (ie, the event will not be reported as separate AE). During the course of any AE, the highest severity rating will be reported.

Deviations from the protocol-specified dosage (including overdosing, underdosing, abuse, and withdrawal), treatment errors (including incorrect route of administration, use of an incorrect product, and deviations from the protocol-defined dosing schedule), 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.

If an investigator becomes aware of an SAE occurring in a subject after study completion, the SAE must be reported on the provided SAER form within 24 hours after awareness; no additional reporting on eCRFs is necessary.

11.1.2.1 Severity

The investigator will assess the severity of each AE using his/her clinical expertise and judgment based on the most appropriate description below:

1. Mild
   - The AE is a transient discomfort and does not interfere in a significant manner with the subject's normal functioning level.
   - The AE resolves spontaneously or may require minimal therapeutic intervention.
2. Moderate
   - The AE produces limited impairment of function and may require therapeutic intervention.
   - The AE produces no sequela/sequelae.
3. Severe
   - The AE results in a marked impairment of function and may lead to temporary inability to resume usual life pattern.

- The AE produces sequela/sequelae, which require (prolonged) therapeutic intervention.

These severity definitions will also be used to assess the severity of an AE with a study-related procedure(s), if necessary.

11.1.2.2 Causality

Causality is a determination of whether there is a reasonable possibility that the IP is etiologically related to/associated with the AE. Causality assessment includes, eg, assessment of temporal relationships, dechallenge/rechallenge information, association (or lack of association) with underlying disease, presence (or absence) of a more likely cause, and physiological plausibility. For each AE, the investigator will assess the causal relationship between the IP and the AE using his/her clinical expertise and judgment according to the following most appropriate algorithm for the circumstances of the AE:

Related: The adverse event is clearly related to the investigational agent/procedure – ie, an event that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that could not be reasonably explained by the known characteristics of the subject's clinical state.

Possibly Related: An adverse event that follows a reasonable temporal sequence from administration of the study intervention follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by a number of other factors.

Not Related: The adverse event is clearly not related to the investigational agent/procedure - ie, another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

For events assessed as not related or unlikely related and occurring within 14 weeks after the IP administration, the investigator shall provide the alternative etiology. These causality definitions will also be used to assess the relationship of an AE with a study-related procedure(s), if necessary.

11.2 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.

Urgent safety measures may be taken by the sponsor or clinical investigator, and may include any of the following:

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

The investigator may take appropriate urgent safety measures in order to protect subjects against any immediate hazard to their health or safety. The measures should be taken immediately and may be taken without prior authorization from the sponsor. In the event(s) of an apparent immediate 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, but within 1 calendar day after the change is implemented. The sponsor will also ensure the responsible EC and relevant competent authority(s) are notified of the urgent safety measures taken in such cases according to local regulations.

11.3 Untoward Medical Occurrences

Untoward medical occurrences occurring before the first exposure to IP are not considered AEs (according to the definition of AE, see Section 11.1). However, each **serious** untoward medical occurrence experienced before the first IP exposure (ie, from the time of signed informed consent up to but not including the first IP exposure) will be described on the AE eCRF and on the SAE Report Form. These events will not be considered as SAEs and will not be included in the analysis of SAEs.

For the purposes of this study, each non-serious untoward medical occurrence experienced by a subject undergoing study-related procedure(s) before the first IP exposure will be recorded on the AE eCRF; these events will not be considered as AEs and will not be included in the analysis of AEs.

For the purposes of this study, each of the following non-serious events experienced after the first IP exposure will not be considered an AE, and thus, not included in the analysis of AEs:

- Hospital or study site visits for administration of BAX 888.
- Hospitalization for routine bleeding episode management that could be managed in the study site or home-setting but for which the subject was hospitalized.
- Hospitalizations for planned medical or surgical procedures, eg, placement of a central venous line.
- Hospitalization or prolongation of hospitalization intended only for social reasons.

- Hospital admittance without inpatient hospitalization or emergency room visit/admittance in itself (although the event triggering the visit may be an SAE).
- Seroconversion after documented HBV vaccination prior to or during the study period.
- 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 (eg, a fall), the injury would not be reported as an AE, unless it resulted in a medical finding other than a bleeding episode (eg, abrasion of skin; fractured tibia). Therefore, **any hemophilia-related event** (eg, hemarthrosis, bruising, hemorrhage) **will not be reported as an AE, but these events will be recorded on the bleeding episode eCRF**. However, hemophilia-related events meeting the criteria for seriousness (eg, a gastrointestinal hemorrhage requiring hospitalization) will be reported as SAEs and described on the SAER form.

11.3.1 Pregnancies in Female Partners of Male Subjects

Male subjects will be instructed through the Informed Consent Form to immediately inform the investigator if their partner becomes pregnant during the study or within 6 months after infusion date. A Pregnancy Report eCRF should be completed by the investigator immediately (ie, no more than 24 hours after learning of the pregnancy) and submitted via the electronic data capture (EDC) system. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male subject exposed to BAX 888. The pregnant partner will need to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the investigator will update the Pregnancy Report eCRF with additional information on the course and outcome of the pregnancy. An investigator who is contacted by the male subject or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician. In the event that the EDC system is unavailable, follow reporting instructions provided for Congenital Anomalies/Birth Defects and Abortions. Any congenital anomaly/birth defect in a child born to a female partner of a male subject exposed to study drug should be classified as an SAE, recorded on the AE eCRF, and reported to the Sponsor immediately (ie, no more than 24 hours after learning of the event). Any abortion performed in response to the identification of possible congenital anomaly/birth defect should be reported in the same fashion.

11.4 Non-Medical Complaints

A non-medical complaint (NMC) is any alleged product deficiency that relates to identity, quality, durability, reliability, safety and performance of the product but **did not result in an AE.** NMCs include but are not limited to the following:

- A failure of a product to exhibit its expected pharmacological activity and/or design function, eg, reconstitution difficulty.

- Missing components.
- Damage to the product or unit carton.
- A mislabeled product (eg, potential counterfeiting/tampering).
- A bacteriological, chemical, or physical change or deterioration of the product causing it to malfunction or to present a hazard or fail to meet label claims.

Any NMCs of the product will be documented on an NMC form and reported to the sponsor within 1 business day. If requested, defective product(s) will be returned to the sponsor for inspection and analysis according to procedures.

11.5 Medical, Medication, and Non-Drug Therapy History

At the Screen 1 visit, the subject's medical history will be described for the following body systems including severity (defined in Section 11.1.2.1) 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 FVIII 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 Month 5 up to study completion/discontinuation (see Table 2).

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.

11.6 Physical Examinations

At the Screen 1 visit, a physical examination will be performed on all major organ systems (ie, 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). At screening, if an abnormal condition is detected, the condition will be described on the medical history eCRF.

At subsequent study visits (as described in Table 2), a targeted physical examination will be performed (ie, 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 Section 11.1.1.5), 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.

Height (in or cm) and weight (lb. or kg) will be measured at the Screen 1 visit. Only weight (lb. or kg) will be measured on Screen 2, Day 0, Month 12, Years 2 to 3 (Months 24, 36, 48, and 60), and at study completion/discontinuation.

11.7 Clinical Laboratory Parameters

Where applicable (see Section 14.7), assessments will be performed at a central laboratory, according to the laboratory manual. Blood samples that remain after study testing is done may be stored and used for additional testing. Samples will be destroyed after a maximum of 5 years from the time the final study report has been completed.

Details of blood sampling volumes are presented in the laboratory manual and master ICF.

The schedule for sample collection for laboratory analysis is described in Section 19.3.

11.7.1 Additional Sample Collection

Backup samples taken and stored short-term may be used for example for re-testing, follow-up of an AE(s) or other test results, and/or assay development. After study testing is completed, 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.

If subjects provide consent, the samples may be made available for research or assay development purposes, otherwise, samples will subsequently be destroyed.

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

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

The coagulation studies will consist of PT, activated partial thromboplastin time, FVIII antigen, FVIII activity level (performed as one-stage assay and as chromogenic assay), FVIII Bethesda inhibitor, international normalized ratio, thrombin time, D-dimer, and fibrinogen activity.

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, GGT, and lactate dehydrogenase (LDH).

The FibroSURE™ (Laboratory Corporation of America, Raritan, NJ, United States) will be measured at screening (Screen 1) and at Month 12.

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

Blood will be obtained for assessment of hematology and clinical chemistry parameters and markers of autoimmune response at the study visits presented in Table 3.

Hematology and clinical chemistry assessments will be performed on ethylenediamine-tetraacetic acid-anticoagulated whole blood and serum, respectively, at the central laboratory. The FVIII 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 3 (ie, Weeks 3-18).

11.7.3 Viral Serology

Viral serology testing will include HIV-1 and HIV-2 antibody, Hepatitis B core antibody, HBsAg, HCV 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 Screen 1 visit, except HIV-1 and HIV-2 antibody which will be performed at the Screen 2 visit. HCV RNA will also be performed at Weeks 1 and 6, and at the Early Study Termination Visit. Any positive test in HBsAg will be repeated using a new blood sample.

11.7.4 Gene Mutation and HLA

Assessment of *F8* gene mutations (FVIII genotyping) and human leukocyte antigen (HLA) genotype (MHC haplotype) will be performed at screening (Screen 1 and Screen 2 visits respectively).

F8 gene mutation will be performed at the University of Bonn and HLA genotype testing will be performed at Central laboratory.

The results will be provided to the sites. The investigator will be responsible for informing the subject of the test results. If results for FVIII gene mutation analysis and HLA genotyping are already available at the study site, this information will be recorded in the eCRF. Nevertheless, the analyses will be performed for the study at the central laboratory regardless.

11.7.5 Immunogenicity

The presence of binding antibodies to FVIII (anti-FVIII antibodies) and binding antibodies to FVIII transgene product (BDD-FVIII), binding and neutralizing antibodies to AAV8 and AAV2, and CMI response to AAV8 and FVIII transgene products based on an ELISPOT assay will be determined at the time points presented in Table 3.

Immunogenicity assessments for the proposed study will include:

- **Neutralizing Antibodies to AAV8 and AAV2**: Study subjects will be screened and monitored for the presence of AAV8 (vector used in this study) specific neutralizing antibodies at a central facility using validated protocol. Subjects will also be monitored for binding and neutralizing antibodies against AAV2 that infects humans with high prevalence.
- **Binding antibodies to AAV8** will be assessed using validated enzyme linked-immunosorbent assays (ELISAs) at a qualified laboratory. Binding antibodies to AAV2 will be monitored using a ELISA-based validated assay.
- **CMI Assessment**: The AAV8 and FVIII specific cell mediated immunity will be assessed using validated interferon-γ (IFN-γ) ELISPOT assays at screening and throughout the study period.
- **Binding antibodies to FVIII and BDD-FVIII** will be determined using validated FVIII-specific ELISA assays. The assays will be performed at a qualified laboratory.
- **FVIII Inhibitor**: Development of antibodies that inhibit FVIII activity (FVIII Inhibitors) will be measured at a central laboratory using validated clot-based assays following the Nijmegen modification.

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 Section 11.1 and Section 11.1.1.1, 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.

11.7.6 Vector Genomes

Serum and bodily secretions will be obtained at the time points presented in Table 3 to assess BAX 888 genomes until 2 consecutive measurements are negative.

Quantitative PCR will be used to detect BAX 888 vector genomes 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.

11.7.7 Assessment of Laboratory Values

The investigator's assessment of each laboratory value will be recorded on the eCRF. For each abnormal laboratory value, the investigator will determine whether the value is considered clinically significant or not. For clinically significant values, the investigator will indicate if the value constitutes an new AE (see definition in Section 11.1, and record the sign, symptom, or medical diagnosis on the AE eCRF), is a symptom or related to a previously recorded AE, is due to a pre-existing disease (described in Section 11.1.1.5), or is due to another issue that will be specified. If the abnormal value was not clinically significant, the investigator will indicate the reason, ie, because it is due to a preexisting disease, due to a laboratory error, or due to another issue that will be specified. Additional tests and other evaluations required to establish the significance or etiology of an abnormal value, 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.

11.8 Vital Signs and 12-Lead Electrocardiogram

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 Screen 2, 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 and 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 Section 11.1) 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.

11.9 Hemophilia Joint Health Score

The Hemophilia Joint Health Score (HJHS) measures the health of joints most commonly affected by bleeding in hemophilia (ie, the knees, ankles, and elbows) (Feldman et al., 2011). The HJHS will be measured on Day 0, Months 12, 24, 36, 48, and 60 post-gene transfer, and at study termination/discontinuation visit.

11.10 Patient Reported Outcomes

Patient reported outcomes, based on relevant questionnaires, will be captured in electronic diaries (see Section 9.5) at the time points presented in Table 2.

The PRO instruments to be measured in this study are described below:

PRO	Assessment	Time of Measurement
HRQoL	Generic: SF-36v2, EQ-5D Disease-specific: Haemo-QoL-A	Screen 2, Months 6, 12, 24, 36, 48, and 60
Patient Experience	Adherence: VERITAS-Pro Disease-specific: HaemoPREF	Screen 2

11.11 Evaluation of Liver Health

Abdominal ultrasound examination will be performed locally at the timepoints presented in Table 2 to assess liver health. In addition, and at the discretion of the Investigator serum alpha fetoprotein level, an exploratory biomarker for hepatocellular carcinoma, will be measured at local laboratories at the time points specified in Table 3.

11.12 Exploratory Assays

Exploratory evaluations of the AAV2 binding and neutralizing antibodies, transcriptome and metabolome as well as whole genome sequencing (if subjects provide additional consent) will be performed to explore the underlying immunologic and other patient-specific features that may affect the transduction and gene delivery by the gene therapy vector (including polymorphisms in immune regulatory genes), the expression and circulation of FVIII, and the changes of the subject's expression of immune-related and other genes before and after gene delivery (T-lymphocyte profiling; transcriptome and genome analysis). These studies will seek to increase the understanding of the variability in gene expression that exists between individuals with hemophilia treated with gene therapy.

11.13 Assessment of Pharmacodynamics

The pharmacodynamic activity of BAX 888 will be evaluated using the following assessments:

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

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

12. STATISTICS

All details regarding the statistical analysis and the preparation of tables, listings, and figures will be described in the statistical analysis plan (SAP).

12.1 Sample Size and Power Calculations

The sample size is expected to range from 2 to 12 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. The sample size and escalation plan are designed to minimize the risk to subjects of experiencing significant toxicity or receiving a sub-therapeutic dose. If a given dose shows lack of efficacy, enrollment will be limited to 2 subjects in the cohort.

With the limited number of subjects per group, statistical analyses will be primarily conducted in a descriptive fashion. There are no inferential procedures planned.

12.2 Analysis Sets

Classification into the Safety Analysis Set will be conducted prior to database lock. The Safety Analysis Set will consist of all subjects that have received 1 administration of the IP. All safety analyses (including the primary analysis) will be performed on the Safety Analysis Set.

12.3 Handling of Missing, Unused, and Spurious Data

Since the statistical analyses will be presented in descriptive summary tables and individual data listings, no action will be taken to handle missing data. All data will be evaluated as observed; no imputation method for missing values will be used. A subject who withdraws prior to the last planned observation in a trial period will be included in the analyses up to the time of withdrawal.

12.4 Methods of Analysis

In general, descriptive summaries will be presented for the primary, secondary, and tertiary outcome measures. Continuous variables will be summarized using mean, standard deviation (SD), 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. The primary outcome of the incidence of BAX 888-related AEs will be analyzed as a categorical variable. More detailed information about summarization of data, graphical representations, and analysis conventions will be provided in the SAP.

12.4.1 Primary Outcome Measure

The number and proportion of subjects experiencing product-related AEs (seriousness and as well as severity) occurring up to 5 years after the BAX 888 administration will be summarized by cohort and overall.

In support of the primary outcome measure, a similar display will be presented by cohort and overall for all AEs (seriousness and severity) occurring up to 5 years after the BAX 888 administration.

An exploratory, descriptive analysis will also be performed for events categorized as development of inhibitory antibodies to FVIII and total binding antibodies to FVIII, severe allergic reactions, and thrombosis-associated events.

12.4.2 Secondary Outcome Measures

12.4.2.1 Efficacy

The secondary efficacy outcome measures (see Section 7.4.2.1) will be assessed using descriptive summaries appropriate for the measure (eg, number and percent for categorical measures and mean, SD, median, minimum, and maximum for continuous measures). For each measure, baseline is the last non-missing reported prior to the infusion of study product.

The change from baseline to each scheduled assessment for FVIII activity level (based on values obtained from the central laboratory one-stage clotting assay) and antigen levels will be summarized for each cohort. The historical ABR as well as the number of post-BAX 888 bleeds, 12 months post-infusion, and 5 years post-infusion will be reported separately for each cohort. The proportion of subjects with a reduction in exogenous FVIII consumption 12 months post-infusion and 5 years post-infusion compared to the historical consumption will be tabulated by cohort.

12.4.2.2 Safety

The secondary safety outcome measures (see Section 7.4.2.2) will be assessed using descriptive summaries appropriate for the measure (eg, number and percent for categorical measures and mean, SD, median, minimum, and maximum for continuous measures).

The number and proportion of subjects will be summarized by cohort for developing the following occurrences at each scheduled assessment: inhibitory antibodies to FVIII (Nijmegen assay) and total binding antibodies to FVIII (IgG and IgM).

12.4.3 Tertiary Outcome Measures

The tertiary efficacy outcome measures (see Section 7.4.3) will be assessed using descriptive summaries appropriate for the measure (eg, number and percent for categorical measures and mean, SD, median, minimum, and maximum for continuous measures).

The HRQoL assessments will be characterized descriptively by cohort for the lead-in period and at the scheduled post-transfusion assessment.

12.5 Planned Interim Analysis of the Study

No formal interim analysis is planned. In addition to a safety data review by the DMC, additional analyses on efficacy and safety data may be performed by the Sponsor at study milestones (eg, when the initial two subjects in the first cohort finishes the Week 18 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.

13. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator/study site will cooperate and provide direct access to study documents and data, including source documentation for monitoring by the study monitor, audits by the sponsor or sponsor's representatives, review by the EC, and inspections by applicable regulatory authorities, as described in the Clinical Study Agreement. If contacted by an applicable regulatory authority, the investigator will notify the sponsor of contact, cooperate with the authority, provide the sponsor with copies of all documents received from the authority, and allow the sponsor to comment on any responses, as described in the Clinical Study Agreement.

14. QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Investigator's Responsibility

The investigator will comply with the protocol (which has been approved/given favorable opinion by the EC), ICH GCP, and applicable regulatory requirements as described in the Clinical Study Agreement. The investigator is ultimately responsible for the conduct of all aspects of the study at the study site and verifies by signature the integrity of all data transmitted to the sponsor. The term "investigator" as used in this protocol as well as in other study documents, refers to the investigator or authorized study personnel that the investigator has designated to perform certain duties. Sub-investigators or other authorized study personnel are eligible to sign for the investigator, except where the investigator's signature is specifically required.

14.1.1 Final Clinical Study Report

All investigators, or the coordinating investigator for multicenter studies, will sign the clinical study report. The coordinating investigator will be selected before study start.

14.2 Training

The study monitor will ensure that the investigator and study site personnel understand all requirements of the protocol, the investigational status of the IP, and his/her regulatory responsibilities as an investigator. Training may be provided at an investigator's meeting, at the study site, and/or by instruction manuals. In addition, the study monitor will be available for consultation with the investigator and will serve as the liaison between the study site and the sponsor.

14.3 Monitoring

The study monitor is responsible for ensuring and verifying that each study site conducts the study according to the protocol, standard operating procedures, other written instructions/agreements, ICH GCP, and applicable regulatory guidelines/requirements. The investigator will permit the study monitor to visit the study site at appropriate intervals, as described in the Clinical Study Agreement. Monitoring processes specific to the study will be described in the clinical monitoring plan.

14.4 Auditing

The sponsor and/or sponsor's representatives may conduct audits to evaluate study conduct and compliance with the protocol, standard operating procedures, other written instructions/agreements, ICH GCP, and applicable regulatory guidelines/requirements.

The investigator will permit auditors to visit the study site, as described in the Clinical Study Agreement. Auditing processes specific to the study will be described in the audit plan.

14.5 Safety Monitoring

This study will be monitored by a DMC. The DMC is a group of individuals with pertinent expertise that reviews on a regular basis accumulating data from an ongoing clinical study. For this study, the DMC will be composed of recognized experts in the field of hemophilia clinical care and research who are not actively recruiting subjects. The DMC can stop a trial if it finds toxicities or if treatment is proven to be not beneficial.

If any one of the following criteria (Cancer Therapy Evaluation Program 2009) is met, further enrollment will be halted until an independent DMC evaluates all available study data and makes a recommendation:

1. Any severe or life threatening AE.
2. Any moderate AE that does not resolve within 14 days.
3. ALT or AST >3 × upper ULN, repeated and confirmed within 72 hours.
4. Development of FVIII inhibitor.

14.6 Non-Compliance with the Protocol

The investigator may deviate from the protocol only to eliminate an apparent immediate hazard to the subject. In the event(s) of an apparent immediate 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, but within 1 calendar day after the change is implemented. The sponsor (Takeda) will also ensure the responsible EC is notified of the urgent measures taken in such cases according to local regulations.

If monitoring and/or auditing identify serious and/or persistent non-compliance with the protocol, the sponsor may terminate the investigator's participation. The sponsor will notify the EC and applicable regulatory authorities of any investigator termination.

14.7 Laboratory and Reader Standardization

Not applicable, a central laboratory will be used for all clinical laboratory assessments.

15. ETHICS

15.1 Subject Privacy

The investigator will comply with applicable subject privacy regulations/guidance as described in the Clinical Study Agreement.

15.2 Ethics Committee and Regulatory Authorities

Before subjects participate in this study, the protocol, ICF, any promotional material/advertisements, and any other written information provided will be reviewed and approved/given favorable opinion by the EC and applicable regulatory authorities. The IB will be provided for review. The EC's composition or a statement that the EC's composition meets applicable regulatory criteria will be documented. The study will commence only upon the sponsor's receipt of approval/favorable opinion from the EC and, if required, upon the sponsor's notification of applicable regulatory authority(s) approval, as described in the Clinical Study Agreement.

If the protocol or other information that is given to the subject is amended, the revised documents will be reviewed and approved/given favorable opinion by the EC and applicable regulatory authorities, where applicable. The protocol amendment will only be implemented upon the sponsor's receipt of approval and, if required, upon the sponsor's notification of applicable regulatory authority(s) approval.

15.3 Informed Consent

Investigators will choose subjects for participation considering the study eligibility criteria. The investigator will exercise no selectivity so that no bias is introduced from this source.

All subjects must sign an ICF before entering into the study according to applicable regulatory requirements and ICH GCP. Before use, the ICF will be reviewed by the sponsor and approved by the EC and regulatory authority(s), where applicable (see Section 15.2). The ICF will include a comprehensive explanation of the proposed treatment without any exculpatory statements, in accordance with the elements required by ICH GCP and applicable regulatory requirements. Subjects will be allowed sufficient time to consider participation in the study. By signing the ICF, subjects agree that they will complete all evaluations required by the study, unless they withdraw voluntarily or are terminated from the study for any reason.

The sponsor will provide to the investigator in written form any new information that significantly bears on the subjects' risks associated with IP exposure. The informed consent will be updated, if necessary.

This new information and/or revised ICF, which have been approved by the applicable EC and regulatory authorities, where applicable, will be provided by the investigator to the subjects who consented to participate in the study.

15.4 Data Monitoring Committee

An external DMC consisting of recognized medical experts in the fields of Hematology, Hepatology, Clinical Immunology, and Gene Therapy as well as one biostatistician will be utilized in this study. This group of individuals with pertinent expertise and who are not active investigators of the current study will review accumulating data on a regular basis. To ensure proper communication between the DMC, study team, ethics committees, and IRBs, procedures will be implemented to provide an exchange of information among the various parties who share responsibility for the successful conduct of the study. Following each DMC meeting, the DMC recommendations will be communicated to Investigators. Specifically, DMC recommendations regarding study continuation with or without modification, dose escalation, and any relevant summary safety information the DMC chair assess as appropriate to include to support the DMC's recommendations will be communicated to Investigators. Questions from Investigators regarding DMC decisions and any responses from the Sponsor regarding DMC-related issues will be communicated to the DMC Chair to ensure that the intent of the DMC is correctly understood by all parties.

16. DATA HANDLING AND RECORD KEEPING

16.1 Confidentiality Policy

The investigator will comply with the confidentiality policy as described in the Clinical Study Agreement.

16.2 Study Documentation and Case Report Forms

The investigator will maintain complete and accurate paper format study documentation in a separate file. Study documentation may include information defined as "source data" (see Section 7.8), records detailing the progress of the study for each subject, signed ICFs, correspondence with the EC and the study monitor/sponsor, screening information, eCRFs, SAERs, laboratory reports (if applicable), and data clarifications requested by the sponsor.

The investigator will comply with the procedures for data recording and reporting. Any corrections to paper study documentation must be performed as follows: 1) the first entry will be crossed out entirely, remaining legible; and 2) each correction must be dated and initialed by the person correcting the entry; the use of correction fluid and erasing are prohibited.

The investigator is responsible for the procurement of data and for the quality of data recorded on the eCRFs. Case report forms will be provided in electronic form.

If eCRFs are provided by the sponsor, only authorized study site personnel will record or change data on the eCRFs. If data is not entered on the eCRFs during the study visit, the data will be recorded on paper, and this documentation will be considered source documentation. Changes to an eCRF will require documentation of the reason for each change. An identical (electronic/paper) version of the complete set of CRFs for each subject will remain in the investigator file at the study site in accordance with the data retention policy (see Section 16.3).

The handling of data by the sponsor, including data quality assurance, will comply with regulatory guidelines (eg, ICH GCP) and the standard operating procedures of the sponsor. Data management and control processes specific to the study will be described in the data management plan.

16.3 Document and Data Retention

The investigator will retain study documentation and data (paper and electronic forms) in accordance with applicable regulatory requirements and the document and data retention policy, as described in the Clinical Study Agreement.

17. FINANCING AND INSURANCE

The investigator will comply with investigator financing, investigator/sponsor insurance, and subject compensation policies, if applicable, as described in the Clinical Study Agreement.

18. PUBLICATION POLICY

The investigator will comply with the publication policy as described in the Clinical Study Agreement.

19. SUPPLEMENTS

19.1 Study Design Flow Chart and Dose Escalation Scheme

Figure 2. Study Design for Takeda Clinical Study 201501

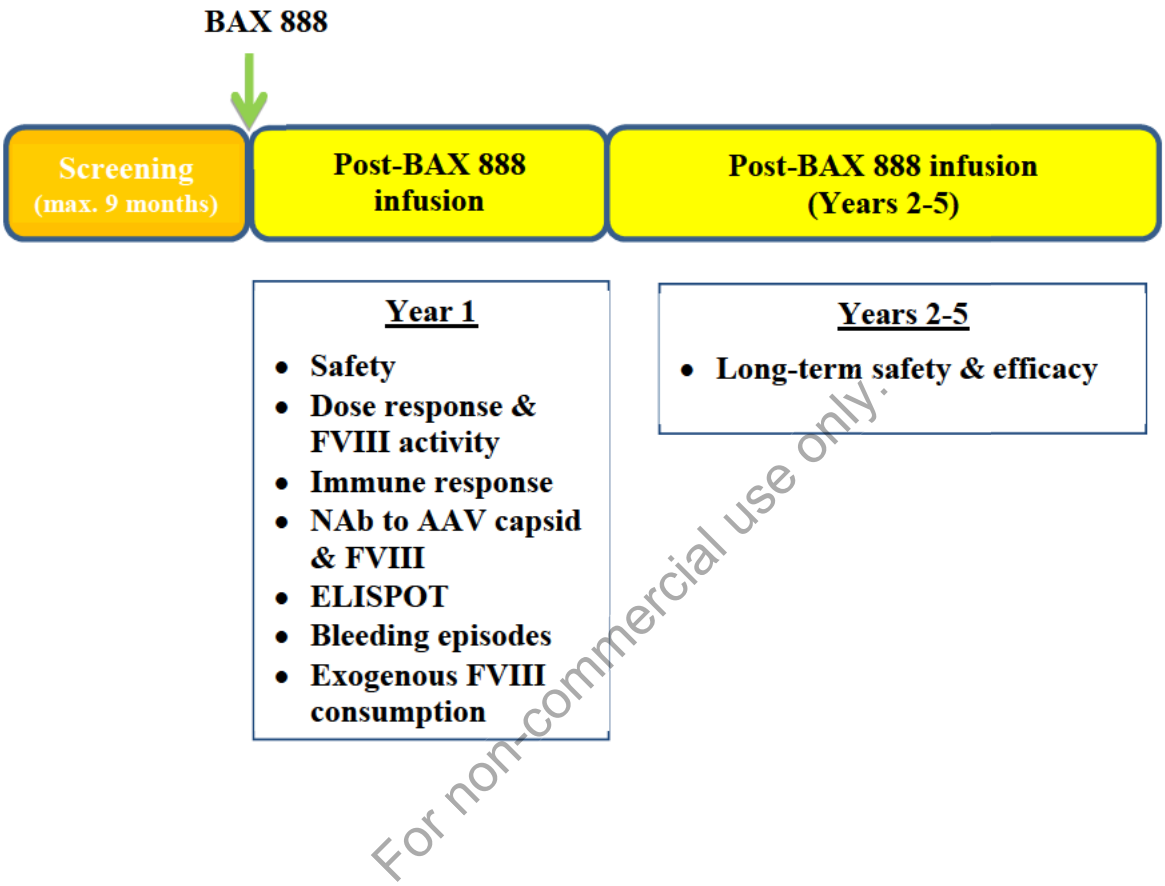
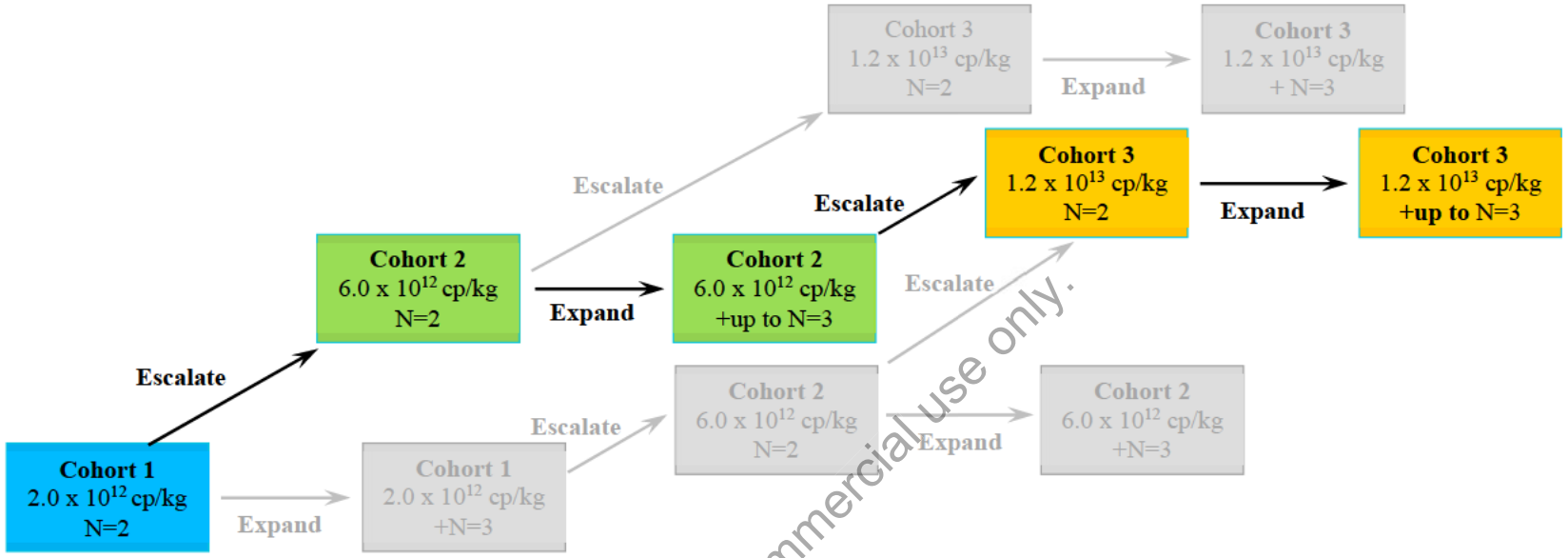


Figure 3. Dose Escalation Schema for Takeda Clinical Study 201501



Dose Escalation / Cohort Expansion Decision Based on <u>Week 4</u> data:

- If Week 4 FVIII levels ≈ baseline (ie,<2%) in the first 2 subjects of Cohort 1, then escalate dose (DMC review not required).
- If Week 4 FVIII levels are ≥2% baseline in at least 1 of the first 2 subjects in any cohort, then dose escalation / cohort expansion decision will be based on data through Week 14.

Dose Escalation / Cohort Expansion Decision Based on <u>Week 14</u> data:

- If sustained Week 14 FVIII activity level of ≥30%[v,viii] is not achieved
  - in the first 2 subjects of Cohort 1 or Cohort 2, then escalate or expand dose after DMC review of all available safety and FVIII activity level data.
  - in at least 3 subjects in Cohort 1 or in Cohort 2, then escalate dose after DMC review of all available safety and FVIII activity level data.
  - in both subjects in Cohorts 3, then dosing of additional subjects will be paused until further review of all available data.
- If sustained Week 14 FVIII activity level of ≥30%[v,viii] is achieved:
  - in at least 1 of the first 2 subjects of any cohort, then expand the cohort with dosing of 3 additional subjects for a total of 5 subjects in that cohort.
  - in at least 3 subjects of Cohort 1 or 1 subject in Cohort 2, then either:
    - Escalate to next dosing cohort
      OR
    - Complete study with no further dosing
      OR
    - Expand the cohort

If any subject in a given cohort achieves FVIII activity levels >50% at Week 14, DMC must review all safety and FVIII activity level data to provide a recommendation on further dose escalations.

19.2 Schedule of Study Procedures and Assessments

Table 2. Schedule of Study Procedures and Assessments

Procedures/ Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[a] (BAX 888 Dose)**	**Day 1**	**Weeks 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[s]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48 and 60**	**Early Study Termination Visit[b]**
Window	Screen 1[u] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[v,] to occur ≤70 days of Day 0	N/A	N/A	±1 day	±1 day for Clinic Visit Laboratory Visit to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
Informed Consent	X[c, q]	X[r]	X[d]								
Eligibility Criteria	X	X									
Medical History[e]	X	X	X		X	X	X	X	X	X	X
Physical Exam	X[f]								Months 24[g] & 36[g]	X	X[g]
Targeted Physical Exam[h]		X[g]	X[g]	X	X	X[t]	X	X[g]			
Vital Signs		X	X[i]	X	X	X[t]	X	X	X	X	X
12-lead ECG		X	X[j]								
Laboratory Assessments[k]	X	X	X	X	X	X	X	X	X	X	X
BAX 888 Administration			X[l]								
Bleeding Episodes		X	X	X	X	X[t]	X	X	X	X	X

Table 2. Schedule of Study Procedures and Assessments

Procedures/ Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[a] (BAX 888 Dose)**	**Day 1**	**Weeks 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[s]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48 and 60**	**Early Study Termination Visit[b]**
Window	Screen 1[u] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[v,] to occur ≤70 days of Day 0	N/A	N/A	±1 day	±1 day for Clinic Visit Laboratory Visit to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
Concomitant Medications (including FVIII)		X	X	X	X	X[t]	X	X	X	X	X
Distribute /collect/ review diary[m]		X	X	X	X	X[t]	X	X	X	X	X
AEs		X	X	X	X	X[t]	X	X	X	X	X
Review Subject's Recent Physical Activity[n]			X	X	X	X[t]	X	X	X	X	X
Unscheduled Visit[o]								X	X	X	
Hemophilia Joint Health Score			X					Month 12	Months 24 & 36	X	X
PROs:[p] <u>HRQoL:</u> • SF-36v2 • EQ-5D • Haemo-QoL-A		X						Months 6 & 12 (with exclusion of	Months 24 & 36 (with exclusion of VERITAS-	X (with exclusion of VERITAS-Pro and	X (with exclusion of VERITAS-Pro

Table 2. Schedule of Study Procedures and Assessments

Procedures/ Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[a] (BAX 888 Dose)**	**Day 1**	**Weeks 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[s]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48 and 60**	**Early Study Termination Visit[b]**
Window	Screen 1[u] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[v,] to occur ≤70 days of Day 0	N/A	N/A	±1 day	±1 day for Clinic Visit Laboratory Visit to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
Patient Experience • Validated Hemophilia Regimen Treatment Adherence Scale – Prophylaxis (VERITAS-Pro) • Disease-specific patient experience (HaemoPREF)								VERITAS-Pro and HaemoPREF)	Pro and HaemoPREF)	HaemoPREF)	and HaemoPREF)
Abdominal ultrasound to assess liver health[w]		X						Month 12	Months 24 and 36	X	

Table 2. Schedule of Study Procedures and Assessments

Procedures/ Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[a] (BAX 888 Dose)**	**Day 1**	**Weeks 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[s]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48 and 60**	**Early Study Termination Visit[b]**
Window	Screen 1[u] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[v,] to occur ≤70 days of Day 0	N/A	N/A	±1 day	±1 day for Clinic Visit Laboratory Visit to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA

a. Subjects will be monitored for the first 8 hours. Following the 8-hour evaluation, at the discretion of the Investigator, the subject and may remain in the infusion center for 24 hours following BAX 888 infusion or return to the center for follow-up at 24 hours post-infusion (Day 1 Study Visit).
b. Includes for cases of withdrawn or discontinuation.
c. Occurs prior to any study-specific procedure.
d. An additional separate consent to be obtained if the infusion center is not the subject's Hemophilia Treatment Center/local investigator's institution.
e. Medical history to include hospitalizations, new malignancy(ies), new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of hematologic disorder, or any other new chronic medical conditions. Screen 1 Visit Medical history to also include hemophilia history (confirmation of diagnosis & severity; presence of any target joints; historical ABR based on documented data within the last 12 months), documentation of all FVIII replacement therapies used within the last 12 months and other medications, hospitalizations within the last 12 months, and surgeries over entire lifetime.
f. Includes height and weight.
g. Includes weight (Screen 2, Day 0, Months 12, 24, 36, 48, and 60).
h. Targeted physical exam includes examination of the liver, skin, and other organ systems as driven by signs, symptoms, and complaints.
i. Time points for Day 0 Vital Signs: (a) pre-infusion: up to 3 hours prior to BAX 888 infusion, and (b) post-infusion: 5 mins (±5 mins), 15 mins (±5 mins), 30 mins (±5 mins), 1 hour (±10 mins), 2 hours (±10 mins), 3 hours (±10 mins), 4 hours (±10 mins), 6 hours (±15 mins), and 8 hours (±15 mins)
j. Time points for Day 0 ECGs: (a) pre-infusion: up to 3 hours prior to BAX 888 infusion, and (b) post-infusion: 1 hour (±10 mins), 6 hours (±15 mins)
k. For laboratory assessments, see Table 3.
l. BAX 888 dose to be determined based on subject's weight obtained at Screen 2 visit.

Table 2. Schedule of Study Procedures and Assessments

Procedures/ Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[a] (BAX 888 Dose)**	**Day 1**	**Weeks 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[s]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48 and 60**	**Early Study Termination Visit[b]**
Window	Screen 1[u] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[v,] to occur ≤70 days of Day 0	N/A	N/A	±1 day	±1 day for Clinic Visit Laboratory Visit to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA

m. Subject electronic diaries (e-diaries) to capture data including concomitant medications, FVIII consumption, bleeding episodes, untoward events, and physical activity related to a bleeding episode. To be reviewed by sites at each visit through Week 4 visit, every 2 weeks thereafter through Month 12 visit, once every month during Years 2-3, and then once every year during Years 4-5.

n. Capture the nature and duration of physical activity performed with 48 hours of study visit.

o. Unscheduled visit(s) encompassing FVIII laboratory assessments to be performed if after Week 18, a >50% decrease in FVIII activity level is observed from the previous visit. Weekly visits to continue until stabilization of FVIII activity levels over 3 weeks.

p. See Section 11.10.

q. Screen 1 Visit ICF: Encompasses all procedures and assessments to be completed at Screen 1 Visit only.

r. Screen 2 Visit ICF: Encompasses all procedures and assessments to be completed at Screen 2 and all subsequent Study Visits.

s. Between Weeks 3-14, subjects will attend twice weekly study visits: (1) Weekly Clinic Visit for all specified procedures and complete assessments and (2) Laboratory Visit for collection of laboratory samples only.

t. To be performed only during the weekly Clinic Visits and not the Laboratory Visits.

u. If the Screen 1 visit occurs >180 days (±7 days) prior to the Screen 2 visit, then the Screen 1 visit assessments may need to be repeated at the discretion of the Investigator and Sponsor.

v. If the Screen 2 visit occurs >70 days of Day 0, then the Screen 2 visit assessments may need to be repeated at the discretion of the Investigator and Sponsor.

w. To be performed locally, may be scheduled on different day, but as close to actual visit as possible

19.3 Clinical Laboratory Assessments

Table 3. Clinical Laboratory Assessments

Laboratory Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[b] (BAX 888 Dose)**	**Day 1**	**Week 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[o]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48, 60)**	**Early Study Termination Visit[a]**
Window	Screen 1[s] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[t] to occur <70 days of Day 0	N/A	N/A	±1 day	±1 day for **Clinic Visit Laboratory Visit** to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
Hematology[c]	X	X	X[d]	X	X	Weeks[q] 3, 5, 7, 9, 11, & 13	Weeks 15 & 17	X	X	X	X
Coagulation[e]	X	X	X[d]	X	X	X[q]	X	X	X	X	X
Clinical chemistries[f]	X	X	X[d]	X	X	Weeks[q] 3, 5, 7, 9, 11, & 13	Weeks 15 & 17	X	X	X	X
Liver Function Test (Central and/or Local Laboratories)		X[p]				X[p]	X				
HIV serology		X									
MHC haplotype[g]		X									
Hepatitis B surface antigen	X										
Hepatitis C antibody	X										
HCV RNA	X				Week 1	Week 6[q]					X
vWF antigen		X				Week 6[q]		Month 6			

Table 3. Clinical Laboratory Assessments

Laboratory Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[b] (BAX 888 Dose)**	**Day 1**	**Week 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[o]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48, 60)**	**Early Study Termination Visit[a]**
Window	Screen 1[s] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[t] to occur <70 days of Day 0	N/A	N/A	±1 day	±1 day for **Clinic Visit Laboratory Visit** to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
FibroSURE[h]	X							Month 12			
Collection of whole blood for transcriptome and plasma/serum for metabolomics analysis			Pre-infusion and 8 h post-infusion[u]	X	X	X[q]		X			X
Markers of autoimmune-mediated hepatitis[i]	X										
Serum cytokines[j]			X[d]	X							
FVIII genotyping	X										
FVIII activity (One-stage and chromogenic assays; central and local laboratories)[k]	X		Pre-infusion	X	X	X[m]	X	X	X	X	X
FVIII antigen	X		Pre-infusion		X	X[q]	X	X	X	X	X

Table 3. Clinical Laboratory Assessments

Laboratory Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[b] (BAX 888 Dose)**	**Day 1**	**Week 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[o]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48, 60)**	**Early Study Termination Visit[a]**
Window	Screen 1[s] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[t] to occur <70 days of Day 0	N/A	N/A	±1 day	±1 day for **Clinic Visit Laboratory Visit** to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA
FVIII inhibitor (Bethesda assay; central laboratory)	X	X			X	X[q]	X	X		X	X
Antibodies to FVIII and FVIII transgene product[l]	X					Weeks[q] 6 & 8		Months 6, 9 & 12	X	X	X
Neutralizing and binding antibodies to AAV8 and AAV2	X	X	Pre-infusion		Week 2	Weeks[q] 4, 8, & 12		X	X	X	X
PBMCs for CMI response to AAV8 and FVIII transgene products	X				X	X[q]	X	Months 5, 6, & 12			X
Vector genomes via PCR in blood, saliva, urine, stool, and semen		X[r]		X	X[n]	X[n,q]	X[n]	X[n]	X[n]	X[n]	X[n]
Urine protein, total	X										
Alpha fetoprotein (optional assessment at local laboratories)								Month 12	Months 24 and 36	X	
Backup samples		X	8h	X	X	X[o]	X	X	X	X	X

Table 3. Clinical Laboratory Assessments

Laboratory Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[b] (BAX 888 Dose)**	**Day 1**	**Week 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[o]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48, 60)**	**Early Study Termination Visit[a]**
Window	Screen 1[s] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[t] to occur <70 days of Day 0	N/A	N/A	±1 day	±1 day for **Clinic Visit Laboratory Visit** to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA

a. Includes for cases of withdrawn or discontinuation.

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. Hematology panel includes: complete blood count (hemoglobin, hematocrit, erythrocytes [i.e., RBC count], and leukocytes [ie, white blood cell count]) with differential (ie, basophils, eosinophils, lymphocytes, monocytes, neutrophils), platelet counts, RBC distribution width, mean corpuscular volume, and mean corpuscular hemoglobin concentration.

d. Time points for Day 0 sample collection: (a) pre-infusion: up to 3 hours prior to BAX 888 infusion, and (b) post-infusion: 30 mins (±5 mins), 4 hours (±10 mins), and 8 hours (±15 mins).

e. Coagulation studies include: prothrombin time, activated partial thromboplastin time, international normalized ratio, thrombin time, D-dimer, fibrinogen activity.

f. 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.

g. 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

h. FibroSURE Score provided based on an algorithm that includes following laboratory results: ALT, alpha-2-macroglobulin, apolipoprotein A1, total bilirubin, gamma glutamyl transferase

i. ANA, Total IgG, anti-smooth muscle antibody, anti-LKM1 titers. To be assessed if no results within 6 months of screening are available.

j. Serum cytokines including IL-6 and TNFα

k. FVIII activity level assessment with one-stage assay will be utilized for all FVIII activity level assessments and will be performed at the central laboratory. Chromogenic assay will be assessed by the central laboratory in addition to the one-stage assay. FVIII activity levels will also be assessed by the local laboratory at all of the indicated time points and at the Clinic and Laboratory Visits (Weeks 3-14). The local laboratory may use either the one-stage FVIII

Table 3. Clinical Laboratory Assessments

Laboratory Assessments	Screening Visits		Study Visits								
	Screen 1	**Screen 2**	**Day 0[b] (BAX 888 Dose)**	**Day 1**	**Week 1-2 Once Weekly Visits** (Clinic Visits only)	**Weeks 3-14 Twice Weekly Visits** (Clinic Visit and Laboratory Visit)[o]	**Week 15-18 Once Weekly Visits** (Clinic Visits only)	**Months 5, 6, 9, 12**	**Years 2-3 (Months 16, 20, 24, 28, 32, 36)**	**Years 4-5 (Months 48, 60)**	**Early Study Termination Visit[a]**
Window	Screen 1[s] to occur <180 days (±7 days) prior to the Screen 2 Visit AND ≥5 days after last FVIII dose	Screen 2[t] to occur <70 days of Day 0	N/A	N/A	±1 day	±1 day for **Clinic Visit Laboratory Visit** to occur 3-4 days after that week's Clinic Visit	±1 day	±4 days	±7 days	±30 days	
Visit Numbers	1	2	3	4	5 - 6	7 - 30	31 - 34	35 - 38	39 - 44	45-46	NA

activity assay or the chromogenic FVIII 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.

l. Neutralizing antibodies to full-length FVIII as well as total binding antibodies to full-length and transgene-derived FVIII will be assessed at each of the specified time points. Testing for neutralizing antibodies to the FVIII transgene product will be triggered only if binding antibodies to the transgene-derived FVIII is observed

m. To be performed during the weekly Clinic Visit **and** the Laboratory Visit between Weeks 3-14 (see also footnote k).

n. Vector shedding assessed at Screen 2, Day 1, weekly at Clinic Visits between Weeks 1-18, and at Months 5,6, 9, 12, 16, 20, 24, 28, 32, 36, 48, and 60, until two consecutive negative results are obtained in the sample.

o. Clinic Visit to include all indicated laboratory assessments; Laboratory Visit to include the local laboratory Liver Function Test, FVIII activity level assessment, and backup sample collection ONLY. Backup sample collection to be performed once a week between Weeks 3-18 during the Laboratory Visit only.

p. Liver Function Test to include: ALT, AST, and GGT; Local laboratory LFTs performed at Screen 2, then twice weekly at Clinic Visit **and** at the Laboratory Visit between Weeks 3-18. Central laboratory LFTs performed at Clinic Visits at Weeks 4, 6, 8, 10, 12, 14, 16, 18.

q. To be performed during the Clinic Visit and not during the Laboratory Visit.

r. Whole genome sequencing will be performed on a Screen 2 sample if subject provides consent.

s. If the Screen 1 visit occurs >180 days (± 7 days) prior to the Screen 2 visit, then the Screen 1 visit assessments may need to be repeated at the discretion of the Investigator and Sponsor. Screen 1 visit assessments that need to be repeated for subjects screened >180 days (±7 days) can be performed in a combined Screen 1 and Screen 2 visit.

t. If the Screen 2 visit occurs >70 days of Day 0, then the Screen 2 visit assessments may need to be repeated at the discretion of the Investigator and Sponsor.

u. 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.

19.4 Definitions

19.4.1 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.

19.4.2 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 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).

20. REFERENCES

Ahmet, A., Kim, H. and Spier, S. 2011. Adrenal suppression: a practical guide to the screening and management of this under-recognized complication of inhaled corticosteroid therapy. *Allergy, Asthma & Clinical Immunology,* 7, 13.

Baldo, A., van den Akker, E., Bergmans, H. E., Lim, F. and Pauwels, K. 2013. General Considerations on the Biosafety of Virus-derived Vectors Used in Gene Therapy and Vaccination. *Current Gene Therapy,* 13, 385-394.

Cancer Therapy Evaluation Program 2009. Common terminology criteria for adverse events (CTCAE).

Committee for Medicinal Products for Human Use 2009. Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products. MEA/CHMP/BPWP/144533/2009., 19.

Crudele, J. M., Finn, J. D., Siner, J. I., Martin, N. B., Niemeyer, G. P., Zhou, S., et al. 2015. AAV liver expression of FIX-Padua prevents and eradicates FIX inhibitor without increasing thrombogenicity in hemophilia B dogs and mice. *Blood,* 125, 1553-1561.

Czaja, A. J. and Manns, M. P. 2010. Advances in the Diagnosis, Pathogenesis, and Management of Autoimmune Hepatitis. *Gastroenterology,* 139, 58-72.

Duncan, N., Kronenberger, W., Roberson, C. and Shapiro, A. 2010. VERITAS-Pro: a new measure of adherence to prophylactic regimens in haemophilia. *Haemophilia,* 16, 247-255.

European Association for the Study of the Liver 2015. EASL Clinical Practice Guidelines: Autoimmune hepatitis. *Journal of Hepatology,* 63, 971-1004.

Fardet, L., Cabane, J., Lebbé, C., Morel, P. and Flahault, A. 2007. Incidence and risk factors for corticosteroid-induced lipodystrophy: a prospective study. *Journal of the American Academy of Dermatology,* 57, 604-609.

Feldman, B. M., Funk, S. M., Bergstrom, B. M., Zourikian, N., Hillard, P., van der Net, J., et al. 2011. Validation of a new pediatric joint scoring system from the international hemophilia prophylaxis study group: Validity of the hemophilia joint health score (HJHS). *Arthritis Care Res,* 63, 223-230.

Kattenhorn, L. M., Tipper, C. H., Stoica, L., Geraghty, D. S., Wright, T. L., Clark, K. R., et al. 2016. Adeno-Associated Virus Gene Therapy for Liver Disease. *Human Gene Therapy,* 27, 947-961.

Liu, D., Ahmet, A., Ward, L., Krishnamoorthy, P., Mandelcorn, E. D., Leigh, R., et al. 2013. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy, Asthma & Clinical Immunology,* 9, 30.

Manno, C. S., Arruda, V. R., Pierce, G. F., Glader, B., Ragni, M., Rasko, J., et al. 2006. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med,* 12, 342-347.

Manns, M. P., Czaja, A. J., Gorham, J. D., Krawitt, E. L., Mieli-Vergani, G., Vergani, D., et al. 2010. Diagnosis and management of autoimmune hepatitis. *Hepatology,* 51, 2193-2213.

Mazepa, M. A., Monahan, P. E., Baker, J. R., Riske, B. K. and Soucie, J. M. 2016. Men with severe hemophilia in the United States: birth cohort analysis of a large national database. *Blood,* 127, 3073-3081.

Nathwani, A. C., Reiss, U. M., Tuddenham, E. G. D., Rosales, C., Chowdary, P., McIntosh, J., et al. 2014. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N. Engl. J. Med,* 371, 1994-2004.

Nienhuis, A. W., Nathwani, A. C. and Davidoff, A. M. 2016. Gene therapy for hemophilia. *Hum Gene Ther,* 27, 305-308.

Perrin, G. Q., Zolotukhin, I., Sherman, A., Biswas, M., De Jong, Y. P., Terhorst, C., et al. 2016. Dynamics of antigen presentation to transgene product-specific CD4+ T cells and of Treg induction upon hepatic AAV gene transfer. *Molecular Therapy.Methods & Clinical Development,* 3, 16083.

Rangarajan, S., Walsh, L., Lester, W., Perry, D., Madan, B., Laffan, M., et al. 2017. AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. *N Engl J Med,* 377, 2519-2530.

Rentz, A., Flood, E., Altisent, C., Bullinger, M., Klamroth, R., Garrido, R. P., et al. 2008. Cross-cultural development and psychometric evaluation of a patient-reported health-related quality of life questionnaire for adults with haemophilia. *Haemophilia,* 14, 1023-1034.

Soloway, R. D., Summerskill, W. H., Baggenstoss, A. H., Geall, M. G., Gitnik, G. L., Elveback, I. R., et al. 1972. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology,* 63, 820-833.

Teal, S., Brohan, E., Hettema, Y., Humphrey, L., Willgoss, T., Hudgens, S., et al. 2014. Development and psychometric evaluation of a novel tool for assessing patient perception and preference for haemophilia treatment (HaemoPREF). *Haemophilia,* 20, 666-673.

van Staa, T. P., Leufkens, H. G. and Cooper, C. 2002. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. *Osteoporos. Int,* 13, 777-787.

World Federation of Hemophilia Treatment Guidelines Working Group 2012. Guidelines for the management of hemophilia 2nd Edition. 80.

Zolotukhin, I., Markusic, D. M., Palaschak, B., Hoffman, B. E., Srikanthan, M. A. and Herzog, R. W. 2016. Potential for cellular stress response to hepatic factor VIII expression from AAV vector. *Mol Ther Methods Clin Dev,* 3, 16063.

21. SUMMARY OF CHANGES

Protocol 201501: Amendment 9 2021 10 NOV

Replaces: Protocol 201501: Amendment 8, 2021 MAR 22

Summary of Changes	
Updated Sponsor information and Protocol History	Title Page Protocol Signature Page Section 14.6 Figure 2 Figure 3