16.1.9 Documentation of Statistical Methods

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Statistical Analysis Plan

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A Phase I/II Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) rh10-Mediated Gene Transfer of Human Factor IX (FIX) in Adults with Moderate/Severe to Severe Hemophilia B

101HEMB01

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List of Abbreviations

AAV rh10	adeno-associated virus serotype rh10
ABR	annualized bleeding rate
ATC	anatomical therapeutic chemical
AE	adverse event
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ATS	as treated set
bCRM	bivariate continual reassessment method
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
eCRF	electronic case report forms
EDA	exploratory data analysis
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot assay
FIX	factor IX
GC	genome copies
ICF	informed consent form
IV	intravenous
MED	minimum efficacious dose
MTD	maximum tolerated dose
NCI	National Cancer Institute
OBD	optimal biological dose
PPS	per-protocol set
PT	preferred term
RNA	ribonucleic acid
SD	standard deviation
SOC	system organ class
SS	safety set
TEAE	treatment-emergent adverse event

1. Introduction

Hemophilia B is an X-linked recessive bleeding disorder that affects approximately 1 in 20,000 to 25,000 male births. The disease is characterized by frequent, spontaneous internal bleeding that can lead to chronic arthropathy (joint damage), intracranial hemorrhage, and even death. It is caused by mutations in the factor IX (FIX) gene leading to deficiencies in coagulation. Depending on the residual activity of FIX, disease severity is classified as mild (>5% to <40% of normal), moderate (1% to 5% of normal), or severe (<1% of normal) [White 2001]. Factor IX gene therapy is expected to be effective for prevention of bleeding subjects with moderate to severe hemophilia B.

Study 101HEMB01 is a dose-finding study. A bivariate continual reassessment method (bCRM) [Braun 2002] is used in this study for the purpose of dose finding. Bearing the characteristics of adaptive design, bCRM not only addresses limitations in the historically simplistic phase I (3+3) design, it also incorporates efficacy information into consideration when identifying dose escalation. Therefore, it is well suited for the rare disease such as hemophilia B where all evaluable information from the patients should be utilized.

This statistical analysis plan (SAP) is based on the clinical study protocol 101HEMB01, version 04 dated 03Sep2015 and its associated electronic case report forms (eCRF). This document describes the rules and conventions to be used in the dose selection, analysis and representation of safety and efficacy data as presented in the clinical protocol.

2. Study Objectives and Endpoints

The study objectives and endpoints are presented in Table 2-1.

Table 2–1 Study Objectives and Endpoints

Objective	Endpoint
Primary	
To determine the safety of single ascending intravenous (IV) doses of DTX101 in adults with moderate/severe to severe hemophilia B.	The incidence of adverse events (AEs), treatment-emergent adverse events (TEAEs), and serious AEs will be summarized for each dosing cohort by severity and relationship to study product.
To establish a dose of DTX101 that achieves a peak plasma level of vector-derived factor IX (FIX) at 6 weeks after IV administration to allow further clinical development.	Peak plasma level of vector-derived factor IX (FIX) at 6 weeks after IV administration as determined by the activated partial thromboplastin time (aPTT) clot-based assay.

Objective	Endpoint
Secondary	
To assess the impact of DTX101 on the number of bleeding episodes requiring recombinant FIX infusion during the study.	The annualized bleeding rate (ABR) will be calculated for all subjects through Week 52 (±7 days).
To evaluate the kinetics, duration, and magnitude of plasma FIX activity, by dose, after IV administration of DTX101 in adults with hemophilia B.	The time course of FIX activity, as determined by aPTT, will be summarized by time point and dose level of DTX101.
To assess the impact of DTX101 on the frequency of FIX replacement therapy during the study.	The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days).
To describe the immune response to the FIX transgene after IV administration of DTX101.	• The development of neutralizing antibodies to FIX (FIX inhibitor), as determined by a Bethesda assay, will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to FIX, as determined by ELISPOT assay, will be summarized by time point and dose level of DTX101.
To assess the impact of DTX101 on the subject's quality of life	Responses to the EQ-5D-5L and Haem-A-QoL questionnaires will be summarized.

Objective	Endpoint
Exploratory	
To describe the immune response to AAVrh10 capsid proteins after IV administration of DTX101.	• The development of neutralizing antibodies to AAVrh10, as determined by ELISA, will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to AAVrh10, as determined by ELISPOT assay, will be summarized by time point and dose level of DTX101. • The development of anti-AAVrh10 binding antibodies, as determined by ELISA, will be summarized by time point and dose level of DTX101.

Abbreviations: AAVrh10, adeno-associated virus serotype rh10; ABR, annualized bleeding rate; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; EQ-5D-5L, EuroQoL 5D 5 level; Haem-A-QoL, haemophilia-specific quality of life.

3. Investigational Plan

3.1. Overall Study Design and Plan

Study 101HEMB01 is a Phase I/II open-label, single arm, multicenter, dose-finding safety study in adults with moderate/severe to severe hemophilia B. The primary objectives of the study are to determine the safety of single ascending IV doses and to identify the Optimal Bilogical Dose (OBD) of DTX101.

3.1.1. Models and Theoretical Background

The OBD, defined as the dose that is less than or equal to the maximum tolerated dose (MTD) and achieves the efficacy target, will be determined by bCRM. Different from the conventional CRM, bCRM takes into consideration both toxicity and efficacy together. Specifically, two regression models,

$$\log\left(\frac{p_{1j}}{1-p_{1j}}\right) = 3 + \beta_1 x_j,$$

and

$$\log\left(\frac{p_{2j}}{1-p_{2j}}\right) = 3 + \beta_2 x_j,$$

are empolyed to describe the relationship bewteen toxicity/efficacy and dose. Here, constant alpha for the toxicity regression model is set up as 3 instead of -3 as shown in Braun's paper because it is demonstrated that the model with -3 performs poorly when the

lowest dose is toxic [Tessela 2005], and 3 is the default setting in Fixed and Adaptive Clinical Trial Simulator.p_{1j} is the probability of toxicity seen at dose j, p_{2j} is the probability of efficacy seen at dose j, and x_j is the nominal dose value, instead of real dose values, derived from prior probability estimate of toxicity and efficacy.

The bCRM is implemented as follows. A first cohort of $n = c$ subjects enters the trial on the dose that is most likely the MTD or the lowest dose. Once information on toxicity and efficacy is collected for all c subjects, the posterior mean of p_{1j}^n and p_{2j}^n can be computed for each dose j. Then we can compute the Euclidean distance

$$d_j^n = \sqrt{\sum_{k=1}^{2}(p_{kj}^n - p_k^*)^2}$$

for each dose j, where p_1^* and p_2^* are the desired rates of toxicity and efficacy. The dose corresponding to the smallest value of d_j^n is selected, and a new cohort of c subjects enters into this dose. Then p_{1j}^n and p_{2j}^n are updated using all information from $n = 2c$. This procedure is repeated until k cohorts ($n = kc$) have been enrolled. The OBD is defined as the dose that minimize the Euclidean distance after all subjects in the trial are evaluated.

A non-informative prior for the regression parameters β_1, β_2 and the association between toxicity and efficacy, ω, are set to have the following form:

$$\pi(\beta_1, \beta_2, \omega) = 6\ \omega(1\text{- }\omega)\ \exp\{-(\beta_1 + \beta_2)\},\ \beta_1 > 0, \beta_2 > 0, 0 < \omega < 1.$$

Here, ω follows a beta (2,2) distribution, β_1,and β_2 follow an exponenetial (1) distribution. The product of beta and exponenetial density functions implies marginal independence of all of three parameters and a mean of 1 for each of the two β's and ½ for ω. Braun [Braun 2002] provides the rationale for this prior: "The prior distribution should be sufficiently defined to offer directions to dose assignment for the first few subjects, but also be sufficiently vague so that the prior distribution's influence on dose assignment decreases as more and more subjects are enrolled."

3.1.2. Dose-Finding Execution

For Study 101HEMB01, a dose limiting toxicity (DLT) is defined as any adverse event (AE)/serious adverse event ≥Grade 3 that is considered to be related to study product by the investigator following National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The MTD is defined as the dose with a posterior probability of experiencing a DLT nearest to 25%.

The peak plasma level of vector-derived FIX at 6 weeks after IV administration, as determined by the activated partial thromboplastin time (aPTT) clot-based assay, is used for modeling the dose-efficacy curve. The target efficacy is peak FIX activity ≥20% of normal level. The minimum efficacious dose (MED) is defined as the dose with a posterior probability of experiencing the target efficacy nearest to 90%.

Subjects will be enrolled sequentially into cohorts of $c = 3$ subjects each. Subjects in the first cohort (Cohort 1) will be assigned to Dose 1(1.6 x 10^{12} genome copies (GC)/kg). Subsequent cohorts will be assigned, on an adaptive basis from bCRM. The following doses selected for evaluation are listed in Table 3–1.

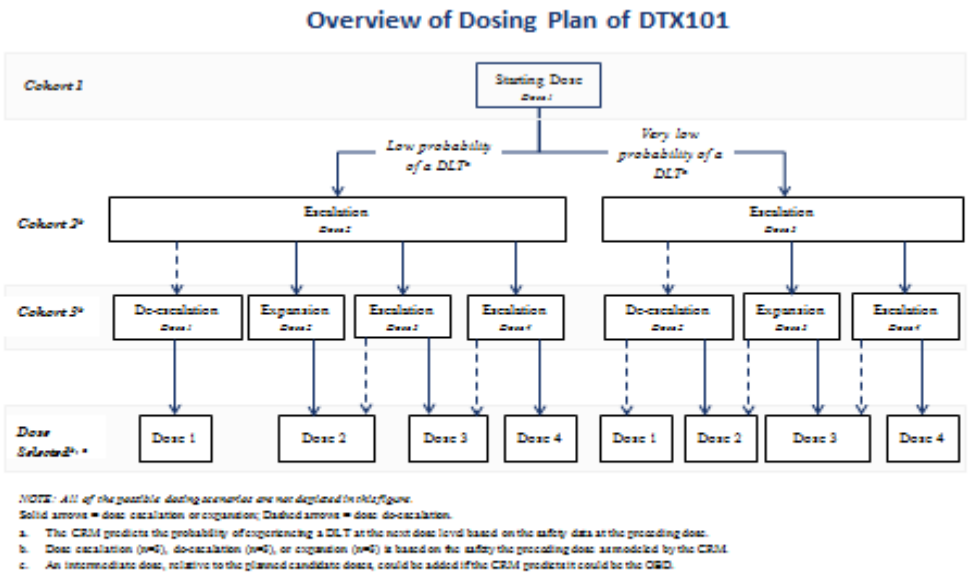
Table 3–1 Candidate Dose of DTX101 to Be Used for Dose-Finding

Candidate Dose	DTX101 Dose Level (GC/kg)
1	1.6×10^{12}
2	3.0×10^{12}
3	5.0×10^{12}
4	1.0×10^{13}

Depending on the results of the bCRM, not all of the candidate doses will be administered. However, the algorithm is allowed to skip at most one dose.

There will be a minimum of 7 days between dosing of each subject within a cohort and a minimum of 42 days between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort.

The dosing algorithm is illustrated by the figure below.



3.2. Treatments

Subjects will receive a single, peripheral IV infusion of DTX101. Subjects in the first cohort will start with the lowest dose and subsequent doses will be guided by the results of the bCRM.

The dose of DTX101 to be administered will be calculated using the subject's weight recorded at screening. The subject's weight will be verified prior to administration of DTX101 to ensure that their current weight is within 10% of their screening weight.

4. General Statistical Considerations for Study Result Summarization

4.1. General Presentation of Summaries and Analyses

Modelling to determine the OBD will be conducted using Fixed and Adaptive Clinical Trial Simulator version 4.0.5 [redacted] All other general data manipulation and statistical analyses will be conducted using SAS Version 9.2 or higher (SAS Institute, Inc., Cary, North Carolina, United States).

Continuous data will be described using descriptive statistics (ie, n, arithmetic mean, standard deviation (SD), median, minimum, and maximum). Categorical data will be described using the subject count and percentage (n, %) in each category. For descriptive statistics of all numerical variables, unless otherwise specified, the minimum and maximum will be displayed to the same level of precision as reported. Mean and median will be displayed to one level of precision greater than the data collected. SD will be

displayed to two levels of precision greater than the data collected. P-values will be rounded to three decimal places. If a p-value is less than 0.001, it will be reported as "<0.001." If a p-value is greater than 0.999, it will be reported as ">0.999."

When count data are presented, the percentage will be suppressed when the count is zero in order to draw attention to the non-zero counts. A row denoted "Missing" will be included in count tabulations where specified in the shells to account for dropouts and missing values. The denominator for all percentages will be the number of subjects within the analysis set of interest, unless otherwise specified.

Change from baseline is the post-baseline value minus the baseline value. If the baseline or post-baseline value is missing, then the change from baseline are set to missing. For each time point where change from baseline is evaluated for continuous variables, descriptive statistics will be displayed for the values at baseline, values at the time point, and values for change from baseline at the time point for the set of subjects who have data at both baseline and the time point being assessed.

Exploratory data analysis (EDA), through descriptive statistics and graphic method, will be conducted for FIX activity throughout the study period.

Imputation will not be performed for all missing values.

Subjects will be identified in the listings by the subject identification number. Data listings will be presented and sorted by subject number, visit, and parameter (where applicable).

Numbering for tables, listings and figures will follow International Conference on Harmonisation E3 Guidelines.

4.2. Analysis Visits

Due to subjects potentially returning outside of the scheduled visits, visit windows will be defined for by-visit summary and analysis purposes.

Only scheduled assessments will be included in the summary tables, which are presented by visit. All assessments (including both scheduled and unscheduled) will be presented in the data listings and figures.

4.3. Definition of Baseline Values and Study Day

Unless otherwise specified, the baseline value for each parameter is defined as the value collected at the time closest to, but prior to, the start of DTX101 administration. The documented history or measurement before Day 0 following the appropriate washout will be used for the baseline values of FIX. For assessments on or after Day 0, the study day will be calculated as assessment date minus date of DTX101 injection (Day 0). For assessments prior to Day 0, the study day will be calculated as the assessment date minus

date of DTX101 injection (Day 0). Study start date is defined as the date the informed consent form (ICF) is signed.

4.4. Sample Size

The study is expected to enroll 3 cohorts of 3 subjects and additional 3 subjects at the OBD for a total of 12 subjects. The maximum of subjects to be enrolled is 18.This sample size consideration is consistent with the sample size of other study with similar design.

4.5. Randomization, Stratification, and Blinding

This is an open label study, so no randomization is implemented.

4.6. Analysis Set

4.6.1. Safety Set (SS)

The SS will include all subjects who receive DTX101. Subjects who have a partial dose will be considered as being treated and included in the SS. The SS is used to tabulate and list all of the safety endpoints.

4.6.2. As Treated Set (ATS)

The ATS will include all subjects who receive any amount of DTX101. Subjects will be treated by the dose they receive. Subjects who receive a partial dose will be adjusted to the nearest candidate dose (see Table 3-1) based on the dose they actually received. ATS will be used for all efficacy analyses and bCRM modelling.

4.6.3. Per-Protocol Set (PPS)

The PPS will consist of all subjects who receive DTX101, have no significant protocol deviations, and have at least 60% of scheduled FIX activity samples collected during the study (52 weeks). Protocol deviations, whether significant or not, will be identified prior to database lock. PPS is used as a supplement to the ATS analysis.

4.6.4. Protocol Deviation Resulting in Exclusion from the PPS

Protocol deviations will be classified as either significant or not. A list of protocol deviations resulting in exclusion from the PPS is provided in Appendix C of the SAP.

5. Subject Disposition

Disposition will be summarized for all enrolled subjects by dosage and overall. It will include the number and percentage of subjects in each analysis set (SS, ATS, and PPS). The number and percentage of subjects who have completed, are ongoing or discontinued from the study as well as reason for study discontinuation will be tabulated. Note that any set-specific summaries should be based on the subjects within that set.

The reason(s) for study discontinuation will be presented as a data listing and may include any of the following: withdrawal of consent, administrative decision by the investigator or the sponsor, ineligibility, significant protocol derivation, subject noncompliance, and AE.

6. Demographics and Baseline Characteristics

6.1. Demographics

Baseline demographic data to be evaluated will include age, sex, ethnicity, race, height and weight. Age will be calculated as the integer part of (ICF Date - Birth Date + 1)/365.25. All demographic and baseline characteristic data will be listed for each subject in the SS. No inferential statistics will be generated.

6.2. Medical History

Medical history will be presented as data listing. The listing will present all patients in the SS.

6.3. Hemophilia B History

Hemophilia B history will be presented as data listing. The documented FIX activity and number of bleeds in the past 12 months will be displayed by descriptive statistics.

The data listing will present year of diagnosis of Hemophilia B, start year of Hemophilia B therapy, family history of Hemophilia B, documented FIX activity, treatment type, number of bleeds in the past 12 months and treatment medicine information. The table and listing will present all patients in the SS.

6.4. Inclusion and Exclusion Criteria

All inclusion/exclusion information on enrolled subjects will be presented in a data listing. The listing will include the failed inclusion/exclusion criteria. A table summarizing the failed inclusion/exclusion criteria will be presented for enrolled subjects.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Medications will be coded using the World Health Organization Drug Dictionary (Version Sep 2015). If the start or end date of medications is missing, it will be imputed as shown in Appendix A. If the start date is completely missing, and the end date of a medication is prior to the initiation of study treatment (Day 0), it will be counted as a prior medication. If the start date is completely missing, and the end date of a medication is after the initiation of study treatment (Day 0), it will be counted as a concomitant medication. If the end date is completely missing, it will be counted as a concomitant medication.

7.1.1 Prior Medications

Prior medications are defined as those medications with a recorded end date prior to the initiation of study treatment (Day 0). Prior medications will be presented as a data listing for all patients in SS.

7.1.2 Concomitant Medications

Concomitant medications are defined as those medications that are taken on or after the initiation of study treatment (Day 0).

Concomitant medications will be presented as a data listing for all patients in SS.

7.2. Drug Exposure

The duration of study participation will be summarized by dosage for all subjects. The duration of study participation (days) is calculated as the date of the last visit (as recorded on the Study Completion/Early Withdrawal page) minus date of the Day 0 visit plus 1. If the date of the last visit on the Study Completion/Early Withdrawal page is missing, or if a subject is lost to follow-up, the latest available visit date will be used. The duration of study participation for each subject will also be presented in a listing.

The SS will be used for all analyses defined above.

8. Efficacy Analysis

The primary efficacy endpoint is defined as the change from baseline in FIX activity at Week 6 as determined by aPTT. The EDA, through descriptive statistics and graphic method, will be performed on the primary efficacy endpoint.

The secondary efficacy endpoints include: ABR; and the annualized and average weekly FIX replacement therapy. All secondary efficacy endpoints will be presented in a listing at the scheduled time points for each subject and will be summarized by dosage at scheduled time points. No formal statistical inference will be performed for the secondary efficacy endpoints.

All efficacy analyses will be conducted on the ATS. Additionally, sensitivity analyses for the primary efficacy endpoint will be performed by utilizing the PPS; additional sensitivity analyses will be performed if necessary.

9. Safety Analysis

Safety evaluations will be based on the incidence, severity, type of AEs, clinically significant changes or abnormalities in the subject's vital signs, and clinical laboratory results.

All safety analyses will be conducted on the SS.

9.1. Adverse Events

Adverse event terms recorded by the clinical site will be mapped to PT using MedDRA, (version18.1). Severity/toxicity grade will be defined according to the NCI CTCAE v4.03.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study (from the time the subject signs the ICF until their exit from the study) regardless of its causal relationship to study treatment. A TEAE is defined as any event not present before exposure to study product or any event already present that worsens in severity or increases in frequency after exposure to study product.

The relationship of TEAE to study product is categorized as “unrelated”, “possibly related”, “probably related”, or “definitely related”. For summaries by relationship, AEs with a missing relationship are considered to be “possibly related”. For summaries by CTCAE grade (grade 1, grade 2, grade 3, grade 4, and grade 5), AEs missing a CTCAE grade are considered to be CTCAE grade 3. If AE start date is missing, it will be imputed as shown in Appendix A.

The overall number of TEAEs will be summarized for the following:

- All TEAE,
- All serious TEAEs,
- All TEAEs with grade >=3
- All study product related TEAEs,
- All study product related TEAEs >= grade 3,
- All study product related serious TEAEs,
- TEAEs leading to study discontinuation,
- Any TEAE leading to death.

The total number, the frequency, and the percentage of subjects with TEAEs will be tabulated by dosage, SOC, and PT for the following:

- TEAEs,
- Serious TEAEs,
- TEAEs by relationship,
- TEAEs resulting in study discontinuation,
- TEAEs by severity,
- Related TEAEs,
- Related serious TEAEs,
- TEAEs grade >=3,
- All study product related TEAEs/Serious TEAEs (DLT) >= grade 3.

At each level of summarization, a subject will be counted only once (with the strongest relationship and the greatest severity) for each TEAE he experiences within that level. Data listings will be provided for all TEAEs.

9.2. Death

All subjects who have an AE with an outcome of "Death" will be presented in a listing.

9.3. Clinical Laboratory Evaluations

Hematology, clinical chemistry, urinalysis, and some special laboratory data will be displayed for each scheduled assessment. Hematology will include complete blood count with differential. Clinical chemistry will include sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatinine kinase, bilirubin (total and indirect), alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and lactate dehydrogenase. Urinalysis will include specific gravity, pH, glucose, protein, blood (by dipstick), ketone (by dipstick), and microscopic examination (if blood or protein is found). Other laboratory parameters will include hepatitis B virus surface antigen, hepatitis C virus ribonucleic acid (RNA), and human immunodeficiency virus RNA, prothrombin time/international normalized ratio, fibrin degradation product (D-dimer), and aPTT.

Numeric hematology, clinical chemistry and urinalysis results will be presented in listings. Clinical chemistry is also summarized by dosage using standardized results and change from baseline. ALT will be provided as a separate listing to monitor the ALT level and its relationship with administration of prednisolone and change of FIX activity. A similar figure will also be presented.

9.4. Vital Sign Measurements

. All vital sign data, including heart rate, systolic blood pressure, diastolic blood pressure and respiratory rate for subjects in the SS, will be presented by-subject in a listing.

9.5. Electrocardiogram

ECG data will be presented in a data listing.

9.6. Factor IX Inhibitor

Samples will be collected at scheduled time points to monitor for an immune response to FIX. FIX inhibitor will be presented by-subject in a listing. FIX inhibitor will also be presented over time in a figure for each subject.

9.7. Neutralizing Antibodies to Adeno-Associated Virus rh10

Samples will be collected at scheduled time points to monitor for an immune response to AAVrh10. Antibodies to AAVrh10 will be presented by-subject in a listing. It will also be presented over time along with cell-mediated immune response in a figure for each subject.

9.8. Viral shedding

Saliva, urine, and stool will be collected at scheduled time points to monitor for the presence of shed virus. Viral shedding will be presented by-subject in a listing. It will also be presented over time in a figure for each subject.

9.9. Cell-mediated immune response

The presence of cytotoxic T lymphocytes specific for AAVrh10 and FIX will be determined by ELISPOT assay at scheduled time points. Cell-mediated immune response will be presented by subject in a listing.

9.10. Adeno-associated virus rh10 binding antibody IgG assay

Circulating anti-AAVrh10 antibodies will be determined by ELISA assay at scheduled time points. Circulating anti-AAVrh10 antibodies will be presented by subject in a listing. It will also be presented over time in a figure for each subject.

9.11. Factor IX genotyping

A listing will be provided for the subjects who provide the samples for genotyping.

10. Quality of Life (QoL) Analyses

Two questionnaires, EQ-5D-5L and Haem-A-QoL, will be collected at baseline, week 24, 36, 48, and 52. For EQ-5D-5L Listings will be presented for each questionnaire.

11. Pharmacodynamics

FIX activity, as measured by aPTT will be presented in a listing for each scheduled time points for each subject. FIX activity will be summarized by dosage at each scheduled time point. It will also be presented in a figure along with cell-mediated immune response for each subject. The association between FIX activity and dose will be evaluated by a linear mixed-effects regression model adjusted for potential confounders.

12. Statistical considerations for Data Safety Monitoring Committee (DSMC) meeting

DSMC members will meet after week 6 data from all subjects of a cohort is available. Dosage recommendation computed through bCRM along with necessary tables/listings/figures (denoted by * in the table of contents) will be prepared and presented.

13. Interim Analysis

An interim analysis will be conducted after week 6 FIX activity results are available for all enrolled subjects. All tables/listings/figures for the full analysis will be produced for interim analysis.

14. References

Braun TM. The bivariate continual reassessment method: extending the CRM to phase I trials of two competing outcomes. Control Clin Trial. 2002;23:240-56.

Peyvand F, Garagiola I, Seregni S. Future of coagulation therapy replacement. J Thromb Haemost 2013;11(Suppl 1):84-98.

Simpson ML, Valentino LA. Management of joint bleeding in hemophilia. Expert Rev Hematol. 2012;5(4):459-68.

White GC 2nd, Rosendaal F, Aledort LM, et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis.

Tessella 2005. Simulation Test Results. Bivariate Continuous Reassessment Method.

15. Appendices

Appendix A: Imputation Algorithm for Partial and Missing Dates

Adverse Events

- If the onset date is completely missing, then the onset date is set to date of administration of study drug.
- If year is present and month and day are missing:
    - If year = year of administration of study drug, then set onset month and day to month and day of administration of study drug
    - If year < year of administration of study drug, then set onset month and day to December 31st.
    - If year > year of administration of study drug, then set onset month and day to January 1st.
- If month and year are present and day is missing:
    - If year=year of administration of study drug and
        - If month = month of administration of study drug then set day to day of administration of study drug

- If month < month of administration of study drug then set day to last day of month
- If month > month of administration of study drug then set day to 1st day of month

▪ If year < year of administration of study drug then set day to last day of month
▪ If year > year of administration of study drug then set day to 1st day of month

- For all other cases, set onset date to date of administration of study drug

Concomitant Medications

- If start date is completely missing then start date will not be imputed.
- If year is present and month and day are missing then set start month and start day to January 1.
- If start year and start month are present and start day is missing then set start day to 1st day of month.
- If end date is completely missing then end date will not be imputed.
- If year is present and month and day are missing then set end month and end day to December 31.
- If end year and end month are present and end day is missing then set end day to last day of the month.

Appendix B: Schedule of study

	Period	**Screening**	**Dosing**	**Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see** Error! Reference source not found.**])**							**Unscheduled**	**End of Study/Early Withdrawal**
	Week	**Days −30 to −1**	**Day 0**	**2**	**4**	**6**	**8**	**10**	**12**	**16-48[a]**		**52**
Procedure	**Visit Window (Days)**	**-**	**-**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±4**	**-**	**±7**
Informed consent		X										
Demographics		X										
Medical history		X										
Hemophilia history		X										

Procedure	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Error! Reference source not found.])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Prior medication / therapies / procedures		X										
Review of eligibility criteria		X										
FIX genotyping		X										
HLA genotyping		X										
HBV, HCV, and HIV status		X										
FIX inhibitor (Bethesda assay)		X	X[b]			X	X			X[c]	X[d]	X
Coagulation panel (PT/INR, D-dimer assay, aPTT)		X	X[b]	X	X	X	X	X	X	X	X[d]	X
AAVrh10 neutralizing antibody test (ELISA)		X	X[b]			X	X			X[c]	X[d]	X
Cell-mediated immune response to AAVrh10 and FIX (ELISPOT assay)			X[b]			X	X			X[c]	X[d]	X
AAVrh10 binding antibody IgG assay (ELISA)		X	X[b]			X	X			X[c]	X[d]	X
Saliva, urine, and stool for viral shedding (qPCR)[f]			X[e]	X	X	X	X	X	X	X	X[d]	X
FIX activity (aPTT clot-based assay)		X	X[b]	X	X	X	X	X	X	X	X[d]	X
Hematology / clinical chemistry[g]		X	X[h]	X	X	X	X	X	X	X	X[d]	X

Procedure	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Error! Reference source not found.])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Urinalysis		X		X	X	X	X		X		X[d]	X
12-lead ECG		X	X[i]									X
Vital sign measurements (heart rate, blood pressure, respiratory rate)		X	X[j]	X	X	X	X	X	X	X	X	X
Height and weight[k]		X	X[b, l]									X[l]
Complete physical examination		X										X
Targeted physical examination[m]			X[b]			X			X	X[n]		
AE/SAE monitoring		X	X	X	X	X	X	X	X	X	X	X
Concomitant medications / therapies / procedures			X[b]	X	X	X	X	X	X	X	X	X
Diary distributed			X									
Diary reviewed				X	X	X	X	X	X	X	X	X
Diary returned												X
Record of spontaneous bleeding episodes		X	X[b]	X	X	X	X	X	X	X	X	X
Record recombinant FIX use		X	X[b]	X	X	X	X	X	X	X	X	X
Prophylactic recombinant FIX washout		X[o]				X[p, q]				X[r, s]		X[r, s]
EQ-5D-5L QoL questionnaire			X[b]							X[n]		X

Procedure	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Error! Reference source not found.])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Haem-A-QoL questionnaire			X[b]							X[n]		X
DTX101 infusion			X									

Abbreviations: AAVrh10, adeno-associated virus serotype rh10; AE, adverse event; aPPT, activated partial thromboplastin time; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; EQ-5D-5L, EuroQoL 5D 5 level; FIX, factor IX; Haem-A-QoL, Haemophilia-Specific Quality of Life; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IgG, immunoglobulin G; INR, international normalized ratio; PT, prothrombin time; QoL, quality of life; qPCR, quantitative polymerase chain reaction; SAE, serious adverse event.

a. Following the Week 12 visit, subjects will return to the study site once every 4 weeks starting at Week 16.

b. To be collected before DTX101 administration.

c. Samples to be obtained at Weeks 16, 32, 40, and 48.

d. A laboratory parameter may be repeated if there is any concern about the values obtained. Laboratory values must be repeated if ≥2-fold the upper limit of normal or ≤0.5-fold the lower limit of normal.

e. Saliva and urine samples for viral shedding to be collected before DTX101 administration. Subjects will be provided with an appropriate container to collect a stool sample at home prior to the visit.

f. Samples for viral shedding to be collected at Weeks 2, 4, 6, 8, 10, and 12 and on Days 8, 20, 36, 48, 64, and 76 (see **Error! Reference source not found.**) until negative on 3 consecutive occasions for each sample matrix.

g. Hematology to include: complete blood count and differential. Clinical chemistry to include: sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase, bilirubin (total and indirect), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and lactate dehydrogenase.

h. Samples for clinical laboratory assessments to be collected before DTX101 administration and 0.5, 4, and 8 hours after the start of the infusion.

i. A 12-lead electrocardiogram to be performed in triplicate before the start of infusion. A single 12-lead electrocardiogram to be performed 1 hour after the start of the infusion. The ECGs should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand.

j. Vital signs to be measured at predose, at 5 minutes after the start of the infusion, and at 0.5, 1, 2, 4, 6, and 8 hours after the start of infusion (±5 minutes). The predose measurement of heart rate and blood pressure is to be performed in triplicate, with measurements taken 2 minutes apart. Vital signs will also be measured at 22 hours (±1 hour) after the start of infusion, prior to subject discharge. Vital signs

should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand.

k. Height to be collected at screening only.

l. Only weight to be collected.

m. Targeted physical examination to include assessment of the skin and the respiratory, cardiovascular, and gastrointestinal systems.

n. To be recorded at Weeks 24, 36, and 48.

o. This is only applicable if a subject does not have a documented history of FIX activity and a baseline sample is needed to confirm severity of hemophilia B. For subjects taking long-acting recombinant FIX prophylactically, washout is to start at Day –28 and last at least 21 days (Day –7). For subjects taking traditional recombinant FIX prophylactically, washout is to start at Day –14 and last at least 7 days (Day –7). The baseline sample must be obtained and results available prior to dosing on Day 0.

p. For subjects who take long-acting recombinant FIX prophylactically, start washout at Week 3 for the Week 6 FIX measurement.

q. For subjects who take traditional recombinant FIX prophylactically, start washout at Week 5 for the Week 6 FIX measurement.

r. For subjects who continue to take long-acting recombinant FIX prophylactically, start washout at Week 21 for the Week 24 FIX measurement and again at Week 49 for the Week 52 FIX measurement.

s. For subjects who continue to take traditional recombinant FIX prophylactically, start washout at Week 23 for the Week 24 FIX measurement and again at Week 51 for the Week 52 FIX measurement.

Appendix C: List of Protocol Deviations