Medexus Pharma, Inc.

STATISTICAL ANALYSIS PLAN

PROTOCOL *APVO101-903*

Evaluation of a Recombinant Factor IX Product, APVO101, in Previously Treated Pediatric Patients with Hemophilia B

Protocol Code:	APVO101-903
SAP Version:	Final, version 2.1
SAP Date:	28-MAR-2023
Author:	[redacted]

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1. LIST OF ABBREVIATIONS

ABR	Annualized bleed rate
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
bp	Base pairs
bpm	Beats per minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Counts
CHOP	Chinese hamster ovary cell proteins
eCRF	Electronic case report form
ED	Exposure day
F1+2	Fragment 1+2
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
PT	Preferred Term
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System Organ Class
SMC	Safety Monitoring Committee
SD	Standard Deviations
TAT	Thrombin-antithrombin complex
TEAE	Treatment Emergent Adverse Event
Q1/Q3	Quartile 1/Quartile 3

2. DEFINITIONS

Annualized Bleeding Rate (ABR) is defined as number of bleeding episodes per year.

An adverse event (AE) is defined as any untoward, undesirable, or unintended medical occurrence in a clinical trial subject administered APVO101 which does not necessarily have a causal relationship with APVO101. For purposes of this clinical trial, an AE can therefore be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of APVO101, whether or not related to the product.

Examples of **major surgical procedures** (surgery) include synovectomy, knee or hip replacement, total tooth extraction, tonsillectomy and/or adenoidectomy, surgery for intracranial hemorrhage, radial head excision, arthrodesis, ankle surgery, abdominal surgery, herniorrhaphy, or surgery for major muscle bleed management.

A **bleeding episode** will be defined as a unique entry in the subject bleeding diary. In the event that a bleeding episode occurring at multiple locations is recorded as multiple entries in the bleeding diary, these will be considered as one bleeding episode provided that all entries have the same start date.

Bleeding episodes severity:

- Minor: e.g., uncomplicated hemarthroses and superficial muscle (except iliopsoas) with no neurovascular compromise, other soft tissue bleeds
- Moderate: e.g., hemarthrosis of longer duration, recurrent hemarthrosis, mucous membranes, deep lacerations, hematuria
- Major or life-threatening: e.g., iliopsoas, deep muscle with neurovascular injury, substantial blood loss, central nervous system, pharyngeal, retropharyngeal, retroperitoneal.

Compliance will be calculated for each subject based on actual and planned number of APVO101 doses in the study. The actual number of doses will be counted from the first dose in the treatment phase up to and including the last APVO101 dose date on study. The number of infusions expected will be calculated for each subject based on their prescribed dosing regimen. If a patient received APVO101 dose twice weekly during whole study compliance will be calculated as following:

Compliance % = 100% *[number of infusions recorded in the subject diary/ (date of last dose for prophylaxis – date of first dose)*2/7]

if a patient first received APVO dose twice weekly and then dose frequency was adjusted to once weekly per investigator discretion, compliance will be calculated as following:

Compliance % = 100% [number of infusions recorded in the subject diary/(A + B)], where
A = [2* (date of last dose when patient was on 'twice weekly' frequency – date of first dose)]/7
B = (date of last dose when patient was on 'once weekly' frequency – date of first dose when patient was on 'once weekly' frequency)]/7

If patient dose-frequency was changed more than once, compliance will be calculated similar to the above but taking into account all periods of different dose frequencies in the denominator.

The following **measures of exposure** will be presented for the study:

Parameter	Description
Exposure Day (ED)	APVO101 exposure/infusion. ED will be calculated as the total number of APVO101 infusions during all phases of the study: Pharmacokinetic (PK) [1], Treatment, and Continuation phases, including surgery if applicable. If a subject receives more than one infusion in a single day, all infusions on that day will count as one ED.
Average Exposure per exposure day (IU)	Average APVO101 dose (IU): Sum of all dosages received by a subject (excluding PK doses and dosing during surgery), divided by number of exposure days (excluding PK and surgery)
Average Exposure per exposure day (IU/kg)	Similar to above but for dose in IU/kg
Total Exposure (IU) – PK phase	Planned PK dose IU [2]
Total Exposure (IU/kg) – PK phase	Planned PK dose IU/kg [2]
Total Exposure (IU) –	N/A, no surgeries in the study at the moment of the Statistical Analysis

Surgeries	Plan (SAP) update [3]
Total Exposure (IU/kg) – Surgeries	N/A, no surgeries in the study at the moment of the SAP update [3]
Total Exposure (IU) – All Phases combined including surgeries	Sum of all doses (IU) during the study (PK [1], Treatment and Continuation phases, including surgery if applicable).
Total Exposure (IU/kg) – All Phases combined including surgeries	Similar above but for dose in (IU/kg)

[1] including additional PK during Treatment/Continuation phases
[2] PK during Treatment/Continuation phases should not be included
[3] if surgeries occur in the study by the time of database lock, exposure during surgeries will be derived similar to other total exposure parameters, i.e., as sum of all doses in IU or IU/kg associated with surgery.

3. INTRODUCTION

This SAP covers the statistical analysis and reporting for the protocol APVO101-903 Amendment 2.0 dated 29 Jul 2020, and electronic case report form (eCRF) production version of 23 Sep 2019.

4. STUDY OBJECTIVES

4.1 PRIMARY OBJECTIVES

The following are the primary objectives of APVO101-903:

- To evaluate safety of APVO101 in pediatric subjects with hemophilia B for at least 50 ED;
- To assess efficacy of APVO101 prophylaxis with respect to prevention of breakthrough bleeding and with respect to control of hemorrhaging in pediatric subjects with hemophilia B for at least 50 ED;
- To evaluate PK of APVO101 in pediatric subjects with hemophilia B;
- To evaluate APVO101 immunogenicity response (development of inhibitory and non-inhibitory factor IX binding antibodies and antibodies to Chinese hamster ovary cell proteins [CHOP]).

4.2 EXPLORATORY OBJECTIVES

- To evaluate markers of thrombogenicity [D-dimer, thrombin-antithrombin III complex (TAT) and fragment 1+2 (F1+2)] during the first 24 hours post-infusion of APVO101;
- To evaluate efficacy of APVO101 for perioperative management in pediatric subjects with hemophilia B.

5. STUDY DESCRIPTION

5.1 STUDY DESIGN AND TREATMENT

Study APVO101-903 is a Phase 3/4, single arm, open-label clinical trial. The purpose of the study is to evaluate PK, safety, and efficacy of APVO101 prophylaxis in severe or moderately severe hemophilia B subjects <12 years of age. The study is designed to gather information in 2 age groups of previously treated (with a minimum of 50 previous ED to factor IX replacement therapy) pediatric subjects, specifically those <6 years of age and 6-12 years of age.

Study APVO101-903 consists of 3 distinct phases:

- PK Phase
- Treatment Phase (from first dose after PK phase and up to 50 ED visit)
- Continuation Phase (starting from first dose after 50 ED visit)

Up to 22 subjects will be enrolled in order to have 15 and 20 evaluable subjects to complete the study (i.e., completion of PK assessments and a minimum of 50 ED visit). Treatment with APVO101 to support a surgical procedure (if required) is permitted for subjects in Treatment/Continuation phases of the study.

5.1.1 PK PHASE

PK evaluation will consist of administration of a single 75±5 IU/kg dose, followed by factor IX activity and safety assessments up to 50 hours post-infusion. Dose will be administered after a 4-day washout period from previous factor IX replacement therapy or 3 half-lives washout of a factor IX product with a prolonged half-life.

5.1.2 TREATMENT PHASE

After completion of PK Phase assessments, subjects will be treated with a prophylaxis regimen of APVO101. Subjects will receive a single intravenous (IV) dose of APVO101 twice weekly or at a frequency of infusions as determined appropriate by the investigator for the particular study subject for a total of 50 ED (approximately 6 months). The starting prophylaxis dose will be based on APVO101 recovery from PK Phase assessments (only pre-infusion and 15-30 minute post-infusion samples; ideally within the recommended dose range: 35 – 75 IU/kg, twice weekly).

For treatment and control of breakthrough, spontaneous or trauma-related bleeding episodes, the dose should be based on severity of bleeding episodes; see below:

- Minor bleeding episodes and/or moderate bleeding episodes: 40 – 60 IU/kg;
- Major or life-threatening bleeding episodes: 60 – 100 IU/kg.

During APVO101 prophylaxis treatment and/or APVO101 treatment for control and management of bleeding episodes, every effort will be made to ensure that the prescribed dose per kilogram body weight is given. However, since it may be difficult to determine fractions of a vial for purposes of providing an exact dose, it is acceptable to round up or down to the nearest vial. The process of rounding up or down should not, however, result in the study subject receiving >120% or <90% of the prescribed dose. The exact amount of factor IX infused must be recorded.

5.1.3 CONTINUATION PHASE

Following completion of the Treatment Phase (i.e., 50 ED), the investigator will assess the subject's dose and either assign the most recent dose from Treatment Phase or modify the dose if clinically indicated for an additional ≥50 ED. The Continuation Phase dose should be within the recommended dose range of 35 – 75 IU/kg, twice weekly.

For treatment and control of breakthrough, spontaneous or trauma-related bleeding episodes,

the dose should be based on severity of bleeding episodes; see below:

- Minor and/or moderate bleeding episodes: 40 – 60 IU/kg
- Major or life-threatening bleeding episodes: 60 – 100 IU/kg

5.1.4 SURGERY

In the event of a minor or major surgery during the course of study participation (Treatment/ Continuation Phases), use of either bolus or continuous infusion of APVO101 for surgical coverage is permissible.

Examples of major surgical procedures include synovectomy, knee or hip replacement, total tooth extraction, tonsillectomy and/or adenoidectomy, surgery for intracranial hemorrhage, radial head excision, arthrodesis, ankle surgery, abdominal surgery, herniorrhaphy, or surgery for major muscle bleed management.

The following dosing is recommended for major surgical procedures:

- If using bolus infusions, an infusion of up to 120 IU/kg will be given within 1 hour prior to the start of the procedure, followed by an infusion of approximately 60 IU/kg 12 hours after the first infusion, and up to 120 IU/kg 24 hours after the first infusion, depending on the subject's post-infusion factor IX activity. This regimen of bolus infusions will continue every 12 hours as long as the physician and surgeon deem necessary, but for a minimum of 3 days post-procedure for major surgery or a minimum of 1 day post-procedure for minor surgery.
- If administering a continuous infusion, it will also continue for a minimum of 3 days post-procedure for major surgery or a minimum of 1 day post-procedure for minor surgery. The infusion rate, dose and timing of subsequent infusions and adjustments in dosing will be recorded and guided by factor IX assay results, with the doses of APVO101 administered appropriately to ensure that the plasma factor IX level does not drop below 60%. If continuous infusion is used, the plasma level of factor IX should range between 70% and 110%.

5.2 SAFETY MONITORING COMMITTEE

A Safety Monitoring Committee (SMC) will provide ongoing review of safety data during the trial. Following study initiation, the SMC will meet as needed to review ongoing safety data. The SMC will be responsible for assessing safety and monitoring overall conduct and integrity of the trial. In fulfilling these responsibilities, the SMC may make recommendations concerning continuation and/or stopping of the trial as it relates to safety and risk to the subject population.

6. RANDOMIZATION AND BLINDING

The study is an open-label single-arm study; subjects and members of the clinical study team will not be blinded to treatment.

7. SAMPLE SIZE AND POWER CALCULATION

No formal sample size calculation was performed given that all planned analyses are descriptive in nature. Up to 22 subjects will be enrolled to ensure that at least 15 to 20 evaluable subjects complete the study (i.e., completion of PK assessments and a minimum of 50 ED). The target sample size is intended to enhance the existing pediatric sample size to be compliant with the European Medicines Agency regulatory guidelines and the Food and Drug Administration post-

marketing requirement for APVO101.

8. ANALYSIS ENDPOINTS

8.1 PK ENDPOINTS

PK data will be listed. Detailed descriptions of all PK analyses are provided in a separate PK SAP. Analysis will be included in a separate report.

8.2 EFFICACY ENDPOINTS

8.2.1 PRIMARY EFFICACY ENDPOINT:

ABR while on prophylaxis to prevent bleeding episodes.

8.2.2 SECONDARY EFFICACY ENDPOINTS:

Bleeding episode level:

- Subject rating of efficacy;
- Change in pain;
- Change in swelling;
- Time from onset of bleeding to the first infusion;
- Time from onset of treatment until resolution of the bleeding episode;
- Number of infusions required to treat the bleeding episode.

Subject level:

- Investigator rating of APVO101 prophylaxis efficacy;
- Investigator rating of APVO101 efficacy for control and management of bleeding episodes.

8.2.3 EXPLORATORY EFFICACY ENDPOINTS:

- Surgeon assessment of estimated blood loss at time of surgery
- Surgeon assessment of post-surgery blood loss (at 12-hour and 24-hour post-surgery time points).

8.3 SAFETY ENDPOINTS

- AEs
- Inhibitory factor IX antibodies
- Non-inhibitory factor IX antibodies
- Anti-CHOP antibodies
- Thrombogenicity markers

9. ANALYSIS POPULATIONS

Screened population: All screened subjects.

Safety population: All subjects who received at least one dose of the study drug. This population will be used for all safety and efficacy analyses.

PK population: All subjects who have a valid PK assessment. A PK assessment will be considered valid if quantifiable and evaluable concentration is available.

10. ANALYTICAL PLAN AND STATISTICAL METHODS

10.1 GENERAL CONVENTIONS AND STATISTICAL CONSIDERATIONS

All statistical analyses will be performed and data appendices will be created using the SAS system version 9.4 or higher.

Data collected in this study will be presented in summary tables and subject data listings. Unless otherwise specified, all data will be summarized overall and by age group (<6 years and 6 to <12 years).

Subjects in the younger age group must remain < 6 years age throughout the treatment period (at least 50 ED) and the upper age group must remain < 12 years throughout the treatment period (at least 50 ED).

Descriptive statistics (number of non-missing values, mean, standard deviation [SD], median, first quartile [Q1], third quartile [Q3], minimum and maximum) will be presented for continuous variables. The number of decimal places in mean, median and quartiles will be equal to number of decimal places in the data + 1, decimal places in standard deviation = decimal places in the data + 2, number of decimal places in minimum/maximum will be equal to number of decimal places in the data.

Frequency distributions (counts and percentages) will be presented for categorical variables. If not specified otherwise, the number of observations with non-missing values will be the denominator for percentage calculation. Percentages will include one decimal place except for 100% where no decimal places will be provided.

By-visit and by-study phase summaries will be presented using nominal eCRF visits (which can be up to ±5 ED to actual exposure days, see section 15)

All data collected will be presented in the data listings. Missing parts of the partial dates will be represented with dashes in the listings.

10.2 DEFINITION OF BASELINE, STUDY VISITS, AND VISIT WINDOWS

The last non-missing assessment made prior to the first dose of study drug will be used as the baseline value. The data will be analyzed according to the visits recorded in the eCRF.

10.3 HANDLING OF MISSING DATA

Rules for handling of missing attributes of adverse events are specified in section 10.8.2. Rules for dates imputations are defined below.

10.3.1 DATES IMPUTATIONS

The rules below will be used for classification of AEs as treatment-emergent and medications as concomitant or prior. Data will be presented in the data listings as collected on the eCRF.

The following rules will be applied for AEs and medications with incomplete start dates:

- If partial start date is equal to the corresponding part of the treatment start date then consider the start date equal to the treatment start date,
- If day is missing for start date and month and year are not the same as month and year for the first treatment date, then impute day 1 of the month,
- If day and month are missing for start date and the year is not the same as year for the first treatment date, then impute January 1st of the year.

The following rules will be applied for AEs and medications with incomplete end dates:

- If day is missing for end date and month and year are non-missing, then day will be imputed as last day of the month,
- If day and month are missing for end date and year is non-missing, then day and month will be imputed as December 31st.

AEs and medications without any start and end date information will not be imputed.

The following rules will be applied for incomplete diagnosis date/date of first treatment for bleeding episode:

- If day is missing then impute day 1 of the month
- If day and month are missing then impute January 1st of the year.

10.4 PATIENT DISPOSITION

Subject disposition will be summarized separately for each phase of the study.

The number of subjects screened and reasons for screen failure, will be summarized in the screened population.

The number and percentage of subjects enrolled, completing the study along with reasons for study discontinuation, and number of subjects in each population will be summarized in the Safety population. The number and percentage of subjects who complete 50 ED Visit (Treatment Phase), 100 ED Visit (Continuation Phase) will be summarized in each population and overall.

A listing of study disposition data will be produced.

10.5 PROTOCOL DEVIATIONS

The number and percentage of subjects with at least one major protocol deviation and the categories of the major protocol deviations will be summarized in the Safety population.

Protocol deviations will be classified to major and minor as specified in the Protocol Deviations Management Plan.

All protocol deviations will be provided in a listing.

10.6 PATIENT CHARACTERISTICS

10.6.1 BASELINE AND DEMOGRAPHIC CHARACTERISTICS

Descriptive statistics for continuous variables (age at informed consent [years], height [cm],

weight [kg], and body mass index [kg/m^2]) and frequency counts and percentages for categorical demographic variables (gender, categorized age (<2 years, 2 - <=11 years), race, and ethnicity) will be summarized in the Safety population.

Age at informed consent (years) will be calculated as (informed consent date–date of birth)/365.25.

Baseline and demographics characteristics will be provided in a listing.

10.6.2 MEDICAL HISTORY AND CURRENT MEDICAL CONDITIONS

Disease characteristics will be summarized in the Safety population, and will include descriptive statistics for

- age at hemophilia diagnosis (years)
- time since diagnosis date (months), calculated as (informed consent date – diagnosis date+1)/30.4375;
- time from date first treated for bleeding episode (months), calculated as (informed consent date–date first treated for bleeding episode date+1)/30.4375;
- total number of ED of factor IX replacement therapy

and frequency counts and percentages for:

- severity;
- whether factor IX mutation known (yes/no);
- type of factor IX mutation;
- target joints/body areas;
- swelling (yes/no) and level;
- muscle atrophy (yes/no) and level;
- loss of range of motion (yes/no);
- pronation, supination, flexion, plantar flexion and dorsiflexion levels;
- flexion contracture (yes/no) and level.

Bleeding history will be summarized in Safety population and will include:

- number of bleeding episodes during 6 months prior to screening;
- percentage of spontaneous bleeding episodes;
- number of infusions needed to control the bleeding episode.

If bleeding history is not documented, then estimations provided in the eCRF will be summarized.

Medical history will be summarized in the Safety population and will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) classification and summarized by System Organ Class (SOC), preferred term (PT) and severity grade at screening. Subjects having the same medical condition (based on SOC, PT, and severity grade) more than once will be counted only once for a particular SOC, PT, and severity grade.

All medical history will be listed including medical history itself, hemophilia B history, and bleeding history.

10.6.3 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the latest version of World Health Organization Drug Dictionary.

The number and percentages of subjects with at least one prior non-study factor IX therapy and prior medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 4 and ATC level 2 class.

The number and percentages of subjects with at least one concomitant medication will be summarized by ATC level 4 and ATC level 2 class.

Prior and concomitant medications will be summarized in the Safety population.

Subjects receiving the same medication more than once will be counted only once for a particular medication class. A medication is considered prior if the end date of the medication is prior to the first study drug dose date. A medication is considered concomitant if it is administered after the first study drug administration. Imputation rules specified in Section 10.3 will be applied to partial/missing dates.

Medications having both start and end dates missing will be considered concomitant; in the case of a missing start date and not missing end date the medication will be considered concomitant, unless the end date is prior to the date of the first dose.

All prior and concomitant medications will be provided in a listing.

10.6.4 Concomitant Procedures

All concomitant procedures will be coded using the latest version of MedDRA classification. A procedure is considered concomitant if performed after the first study drug administration.

All concomitant procedures will be provided in a listing.

10.7 Efficacy Endpoints and Analysis

Efficacy data will be analyzed in the Safety population. Descriptive summaries and listings of all Treatment and Continuation Phase efficacy endpoints as described in section 10.7.2, as well as the surgeon's assessment of estimated blood loss during and after surgery, will be provided. The efficacy endpoints are summarized for each study phase (Treatment and Continuation) and combining data across study phases.

10.7.1 Analysis of Primary Efficacy Endpoint

The number of bleeding episodes and the number of months observed will be determined from the subject diary. The months of observation will be calculated from the first date of the Treatment Phase to the last prophylaxis entry into the subject diary.

ABR while on prophylaxis to prevent bleeding episodes will be calculated and summarized using median, Q1, Q3 and 95% exact confidence interval for the mean, assuming that the rates follow a Poisson distribution.

ABR overall = (number of bleeding episodes starting from first dose after PK Phase up to 3 days after the last dose of APVO101 given for prophylaxis)/[(treatment end date +3 – treatment start date+1)/365.25]. If a subject left the study earlier than 3 days after last dose, date of end of study will be taken instead of (treatment end date +3 days).

ABR Treatment Phase = (number of bleeding episodes starting from first dose after PK Phase up to 3 days after the last dose of APVO101 given for prophylaxis up to 50 ED)/[(last dose ≤50ED +3 – treatment start date +1)/365.25]. In case first dose after 50 ED occurred earlier than 3 days after 50 ED then this dose date – 1 should be taken as reference end date. If a subject left the study earlier than 3 days after last dose in treatment phase, date of end of study will be taken instead of (last dose ≤50 ED + 3 days).

ABR Continuation Phase = (number of bleeding episodes starting from first dose after 50 ED up to 3 days after last dose of APVO101 given for prophylaxis)/[(treatment end date +3 – first dose after 50 ED+1)/365.25]. If a subject left the study earlier than 3 days after last dose, date of end of study will be taken instead of (treatment end date +3 days).

Note: a bleeding episode which occurred after PK dose but prior to completion of PK assessment will be included into ABR calculations.

In addition, analysis of ABR will be repeated on the normalized data (square-root transformation will be used to normalize ABR).

Number of subjects with at least one bleeding episode and no bleeding episodes will also be summarized.

The analysis of ABR will be repeated for Spontaneous ABR which will consider only spontaneous bleeding episodes in the numerator. Denominator will remain the same as for ABR. Spontaneous ABR for Treatment, Continuation phases as well as overall will be derived.

10.7.2 Analysis of Secondary Efficacy Endpoints

Both types of secondary efficacy endpoints (bleeding episode level and subject level) will be presented descriptively for the Treatment Phase (bleeding episodes and subject level assessments occurred from first dose to 50 ED up to 3 days after last dose of APVO101 given for prophylaxis), Continuation Phase (bleeding episodes and subject level assessments occurred on or after first dose after 50 ED given for prophylaxis) and overall (all bleeding episodes and subject level assessments in a subject).

Summaries for bleeding episode level endpoints will include:

- Number and percentage of bleeding episodes within each category of subject's rating of efficacy;
- Number and percentage of bleeding episodes within each category reported by subjects as answer to question "How long did it take for the pain to stop?"
- Number and percentage of bleeding episodes within each category of highest level of pain during the bleeding episode.
- Number and percentage of bleeding episodes within each category reported by subjects as answer to question "How long did it take for the swelling to go down?"
- Descriptive statistics for time from onset of bleeding to the first infusion (minutes) as continuous variable

- Descriptive statistics for time from onset of treatment until resolution of the bleeding episode (minutes) as continuous variable
- Descriptive statistics for number of infusions required to treat the bleeding episode as continuous and categorical variable

Note: a bleeding episode which occurred after PK dose but prior to completion of PK assessment will be included into ABR calculations.

Summaries for subject level endpoints will include by-visit summaries for the following Investigator's assessments:

- APVO101 prophylaxis efficacy assessment
- Efficacy for control of bleeding episodes assessment

All data for subject assessments of bleeding episodes and Investigator assessments of efficacy will be listed.

10.7.3 Analysis of Exploratory Efficacy Endpoints:

The following endpoints related to surgery will be summarized as frequency variables:

- Pre- and Post-surgery assessments:
  - Surgeon's assessment of total blood loss

- 12 hours (±3 hours) post-surgery assessment and 24 hours (±3 hours) post-surgery assessment:
  - Surgeon's assessment of total blood loss
  - Surgeon's assessment of APVO101 hemostatic efficacy

All data related to surgeries will be listed including reasons for procedures, prior and post-surgery assessments.

Vital signs collected during surgery will be listed.

10.8 Safety Endpoints and Analysis

All safety outcomes will be analyzed in the Safety population.

Safety will be evaluated by presenting summaries of exposure to study treatment, AEs, physical examination, vital signs, local laboratory evaluations (hematology, serum chemistry, and urinalysis) and central laboratory evaluations (inhibitory factor IX antibodies, non-inhibitory factor IX binding antibodies, anti-CHOP antibodies, and thrombogenic markers).

10.8.1 Exposure to Study Treatment and Compliance

Exposure and compliance parameters will be summarized descriptively. Parameters to be summarized are specified in section 2.

In addition, number and percentage of subjects who fall under the following categories (not mutually exclusive) of treatment exposure will be presented:

- Subjects with ≥ 50 ED
- Subjects with ≥ 100 ED
- Subjects with ≥ 150 ED
- Subjects with ≥ 200 ED

Number of surgeries overall and per subject, number of infusions due to surgeries overall and per surgery, and total dose administered per surgery will be calculated by treatment regimen (bolus vs. continuous infusion); subjects may receive both bolus and continuous infusions and, in such cases, will be included in the summaries for both treatment regimens. For subjects treated with bolus infusion, the number of bolus infusions, average APVO101 dose (IU/kg), and total APVO101 exposure (IU) will be presented by surgery visit (pre-surgery and post-surgery) and overall. For subjects treated with continuous infusion, the average APVO101 dose (IU/kg/hr) and total APVO101 exposure (IU) will be presented.

Compliance derivation algorithm is provided in section 2. Compliance will be summarized as continuous variable as well as categorized into >=80%, < 80 %. Reasons for dose/frequency modifications will also be tabulated.

Summaries described in this section will be done separately for Treatment/Continuation Phases combined, surgeries and overall, except for compliance and reasons for dose/frequency modifications which will be presented for all study duration.

A by-subject listing with the treatment administration data will be presented. In addition, a listing of all derived parameters will be produced, and it will include total value of exposure (IU) and exposure days per study.

Dose regimen data together with reasons for changes in dose regimen will be listed.

10.8.2 ADVERSE EVENTS

AEs will be coded using the latest version of MedDRA. The analysis of AEs will include descriptive statistics and will be summarized through the use of frequency tables overall and by type (AE or serious adverse event [SAE]) SOC and PT, intensity, and relationship of events to treatment. Summaries for adverse events will include number of subjects as well as number of events.

A treatment-emergent adverse event (TEAE) is defined as an AE that occurs or worsens after the first study drug administration. AEs having both onset and end dates missing will be considered as TEAEs. AEs having missing onset date and not missing end date AE will be considered as TEAE unless end date is prior to the date of the first dose.

Related AEs are AEs with missing, probably related, possibly related, and definitely related relationship to study drug.

An overall summary of AEs will include the number of subjects who experienced at least one AE of the following categories: any TEAE; any SAE; any related TEAE, any TEAE leading to early study termination; and any TEAE leading to death.

The incidence of TEAEs will be summarized by SOC and PT; by SOC, PT and intensity; and by

SOC, PT and relatedness. If the same AE (based on SOC and PT) is reported for the same subject more than once, the AE is counted only once for that SOC and PT, and at the highest intensity or relatedness, respectively.

In addition, the incidence of SAEs and TEAEs leading to early study termination will be presented by SOC and PT. If the same AE (based on SOC and PT) is reported for the same subject more than once, the AE is counted only once for that SOC and PT.

Separate listings will be prepared for all AEs, SAEs, AEs leading to early study termination, and AEs leading to death.

10.8.3 LABORATORY DATA

The following types of laboratory assessments will be done in the study:

- Safety laboratory assessments (hematology, chemistry, and urinalysis) will be collected locally
- CD4 markers will be done centrally for certain countries and locally for others
- Anti-CHOP antibodies will be done centrally
- Inhibitory Factor IX antibodies will be done locally and centrally
- Non-inhibitory Factor IX antibodies will be done centrally

Antibodies data from central lab will be summarized along with 95% exact confidence intervals using binomial distribution.

All laboratory assessments (collected locally and centrally) will be listed.

Thrombogenic markers

Thrombogenic markers will be done centrally. Results will be summarized and listed. Values reported to be above or under upper/lower limits of quantitation will be excluded from the summary. In addition, the following tables will be produced

- a summary table where these values (reported to be above or under upper/lower limits of quantitation) will be treated as being equal to upper/lower limit of quantitation.
- a summary table where these values will be treated as being equal to upper/lower limit and excluding a subject whose pre-dose prothrombin fragments 1 + 2 values were significantly higher than post-baseline (see Memo to File, “RE: ZA509-0001 efficacy assessment”,17 Feb 2023).

10.8.4 VITAL SIGNS AND OTHER SAFETY PARAMETERS

10.8.4.1 VITAL SIGNS

Descriptive statistics of vital signs (temperature [degree C], systolic and diastolic blood pressure [mmHg], pulse rate [beats per minute; bpm] and respiratory rate [breaths/min]) at each scheduled visit, as well as the change from baseline will be presented. Summary will be prepared for all assessments excluding those made during surgery. Data collected during surgery will be listed.

All vital signs data will be presented in a by-subject listing.

10.8.4.2 BLEEDING EPISODES

Bleeding episode location, cause and side of bleed will be summarized on by-episode level cumulative at 50ED and 100ED.

10.8.4.3 PHYSICAL EXAMINATION

Listing of physical examination findings will be provided by subject.

10.9 OTHER ENDPOINTS AND ANALYSIS

10.9.1 GENOTYPES

Mutation genotypes of subjects whose mutations are known at screening and those whose who undergo genotyping during the study will be listed. The available genotypes are:

- Missense
- Nonsense
- Synonymous
- Large structure change (>50 base pairs [bp])
- Small structure change (in-frame, <50bp)
- Frameshift
- Promoter
- Splice site change

11. INTERIM ANALYSIS

When a sufficient number of subjects completes their 50 ED visit, available PK and safety/efficacy data (for at least 50 ED) will be included for data analysis of an integrated pediatric report for United States post-marketing requirement.

The summaries presenting all efficacy, safety, and PK endpoints listed in Section 10 will be included into interim analysis.

All data collected in the study by the time of interim analysis will be listed.

12. DEVIATIONS FROM ANALYSIS AS DESCRIBED IN THE PROTOCOL

Protocol version 3.0 amendment 2.0 section 11.4.2. ‘Efficacy analysis specifies:

> *The annualized bleeding rate will be calculated and summarized, including the median, interquartile range and the 95% exact confidence interval for the median, assuming that the rates follow a Poisson distribution.*

Sponsor confirmed that the wrong word has been used in this statement due to oversight. 95% confidence intervals are planned to be derived for mean (not median), analyzed in the same manner as the previous IB1001 studies.

Additional analysis of normalized (square-root transformed) ABR was also added to the efficacy

analysis to provide data comparable to results from studies IB1001-01 and IB1001-02.

According to the protocol, efficacy endpoints are evaluated for the Treatment and Continuation phases of the study (section 11.2.2). The Treatment phase begins after PK phase assessments are completed (section 5.7). The sponsor clarified that the evaluation of APVO101 efficacy with respect to prevention of breakthrough bleeding and with respect to control of hemorrhaging in pediatric patients, should be assessed after the initial infusion of APVO101 (PK phase) as this first infusion of 75 IU/kg is intended to protect subjects prophylactically. Therefore, any bleeding episode that occurs during the PK phase, will be evaluated and considered as belonging to the Treatment phase.

13. PROGRAMMING SPECIFICATIONS

All outputs will be produced using SAS version 9.4 or a later version.

The margins should be at least 1.50 inches for the binding edge and 1.0 inches for all others.

In the top left portion of each output the protocol number and deliverable type will be presented. In the top right the company name and page number will be presented. In the next line 3 lines, centre-adjusted the table/listing number, title and population information will be displayed. Footnotes will be placed under the main body at the bottom of the page. There should be one blank line between table content and the footnotes. The SAS program name and its location and creation date will appear bottom left. The source listing number or analysis data set name will appear on the bottom left corner of each output. The database cut-off/database lock date along with outputs status (Draft/Final) will appear just below the source listing.

Tables and listings (no figures are planned in the study) will be provided to Sponsor as two .pdf files each of them containing all tables and all listings. In addition the same files as .docx files will be provided to PSI medical writing to facilitate CSR writing.

14. LIST OF TABLES AND LISTINGS

Shells for tables and listings are provided in a separate mock-up TLs document.

15. APPENDIX I. SCHEDULE OF ASSESSMENTS

	Screening[12]	PK Phase		Treatment Phase				Continuation Phase			End of Study or Early Termination
Evaluations		**Pre-infusion[1]**	**Post-infusion[2]**	**5 ED (±1 ED)**	**12 ED (±2 ED)**	**25 ED (±3 ED)**	**50 ED (±5 ED)**	**75 ED (±5 ED)**	**100 ED (±5 ED)**	**Every 3 months (~25 ED ±5 ED)**	
Informed consent and/or assent	X										
Eligibility Criteria Review	X										
Medical and hemophilia-related history	X	X									
Demographics (age, sex)	X										
Factor IX mutation assessment					X[10]						
Concomitant medications	X	X		X	X	X	X	X	X	X	X
Physical exam, body weight and height	X	X				X	X	X	X	X	X
Vital signs	X	X	X[3]			X	X	X	X	X	X
Thrombogenic markers[14]		X[14]	X[4,14]								
CBC with differential [5]	X					X[15]	X[15]	X[15]	X[15]	X[15]	X[15]
CD4 count[13]	X										
Serum chemistry[6]	X					X[15]	X[15]	X[15]	X[15]	X[15]	X[15]
Inhibitor titer and non-inhibitory factor IX binding antibodies[9]	X	X[9]		X	X	X	X	X	X	X	X

Evaluations	Screening[12]	PK Phase		Treatment Phase				Continuation Phase			End of Study or Early Termination
		Pre-infusion[1]	Post-infusion[2]	5 ED (±1 ED)	12 ED (±2 ED)	25 ED (±3 ED)	50 ED (±5 ED)	75 ED (±5 ED)	100 ED (±5 ED)	Every 3 months (~25 ED ±5 ED)	
Anti-CHOP antibodies	X	X[16]		X	X	X	X	X	X	X	X
Assessment of major and/or target joints	X						X		X		X
Urinalysis[7]	X					X	X	X	X	X	X
Factor IX activity[11]		X	X								
Adverse events	X		X	X	X	X	X	X	X	X	X
Efficacy/subject diary[8]	X			X	X	X	X	X	X	X	X
Training on subject diary and APVO101 reconstitution and administration	X										

[1] From 2 hours to 15 minutes pre-infusion.
[2] At 15-30 minutes, 4-6 hours, 24-26 hours and 46-50 hours post-infusion of 75 ± 5 IU/kg (PK dose).
[3] Vital signs (pulse rate, blood pressure, respiratory rate and temperature) at 15-30 minutes, 4-6 hours, 24-26 hours and 46-50 hours post-infusion of PK dose.
[4] Thrombogenicity marker assessments at 15-30 minutes, 4-6 hours and 24-26 hours post-infusion of PK dose.
[5] Includes hemoglobin, hematocrit, absolute counts of: white blood cells, neutrophils, monocytes, lymphocytes, eosinophils, basophils, platelets and red blood cells; mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) to be assessed at a local laboratory.
[6] Includes ALT, AST, ALP, BUN or urea, uric acid, creatinine, total bilirubin and glucose to be assessed at a local laboratory.
[7] Urine dipstick, followed by microscopy if abnormal to be assessed at a local laboratory.
[8] Investigator's efficacy assessment and review of subject's diary including infusions, bleeding summary, subject's efficacy assessment, adverse events and concomitant medications. At Screening, review of subject diary to include review of previous factor IX therapy (if available).
[9] Inhibitory and non-inhibitory factor IX antibodies to be assessed by central lab at PK Phase Pre-infusion for subjects weighing ≥ 28 kg, and all subjects at all other time points.
[10] Factor IX mutation assessment to include mutation genotyping by central laboratory if not previously documented. For patients below 10 kg this may be done

at any time during the study when blood volumes allow

[11] Factor IX activity to be assessed by central lab pre-infusion and post-infusion at 15-30 minutes, 4-6 hours, 24-26 hours, and 46-50 hours. Pre-infusion and 15 to 30 minutes post-infusion samples will be tested for factor IX activity to determine the starting prophylaxis dose. See protocol section 5.7.1 for further details.

[12] Screening to occur 21 to 5 days prior to PK phase.

[13] CD4 count to be assessed by local laboratory.

[14] See protocol Table 2 for thrombogenicity assessments by subject weight; thrombogenicity markers include D-dimer, TAT complex, fragment 1+2.

[15] For patients below 17 kg, CBC with differential and serum chemistry should be collected if blood volumes allow.

[16] Anti-CHOP antibodies to be assessed by central laboratory at PK phase pre-infusion for subjects weighing ≥ 28 kg

Table 1: Thrombogenicity Assessments During PK Phase by Subject Weight

Subject weight	Pre-infusion	15-30 min post-infusion	4-6 hours post-infusion	24-26 hours post-infusion
≥ 20 kg	X	X	X	X
≥ 17 kg to <20kg	X	X		X
<17 kg[1]				

[1] To accommodate blood volume limitations for subjects <17 kg, no thrombogenicity assessments will be performed during the PK Phase. Thrombogenicity assessments may be drawn at these timepoints during the Treatment Phase when blood volume limitations allow.

Table 2: Schedule of Events for a Surgical Procedure (if required during Treatment Phase and/or Continuation Phase)

	Prior to surgery[1]		During surgery	End of surgery	12 hours post-surgery		24 hours post-surgery[2]		28 days (±7 days) post-factor IX replacement therapy for surgery
Evaluations	**Pre-infusion**	**Post-infusion (5-30 min)**			**Pre-infusion**	**Post-infusion (5-30 min)**	**Pre-infusion**	**Post-infusion (5-30 min)**	
Factor IX activity[3]	X	X	X[4]		X	X	X	X	
Vital signs	X				X		X		
Adverse events		X	X	X		X		X	

	Prior to surgery[1]		During surgery	End of surgery	12 hours post-surgery		24 hours post-surgery[2]		28 days (±7 days) post-factor IX replacement therapy for surgery
Evaluations	**Pre-infusion**	**Post-infusion (5-30 min)**			**Pre-infusion**	**Post-infusion (5-30 min)**	**Pre-infusion**	**Post-infusion (5-30 min)**	
Use of blood products			X	X	X		X		X
Surgeon's assessment of expected/estimated blood loss	X			X	X		X		

[1] If using bolus infusions, an infusion of up to 120 IU/kg will be given within 1 hour prior to the start of the procedure, followed by an infusion of approximately 60 IU/kg 12 hours after the first infusion and an infusion of up to 120 IU/kg 24 hours after the first infusion. If administering a continuous infusion, the plasma factor IX activity should range between 70% and 110%, and the dose and timing of subsequent infusions and adjustments should be recorded and guided by factor IX activity results.

[2] Continue with bolus or continuous infusion treatment for as long as the surgeon deems necessary, but for a minimum of 3 days post-surgery for major surgery or a minimum of 1 day post-procedure for minor surgery. Assess pre- and post-infusion factor IX activity every 12 hours.

[3] Factor IX activity is to be assessed by the local laboratory at all time points. If blood volumes allow, factor IX samples should also be assessed by the central laboratory.

[4] If study product is infused during surgery, measure factor IX activity pre- and post-infusion (5-30 minutes).