Statistical Analysis Plan

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2 Table of Contents

3 List of Abbreviations

ADR	Adverse drug reaction
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate transaminase
AUC	Area Under the Concentration-Time Curve
BE	Bleeding episode
CL	Apparent clearance
C_{max}	Maximum Plasma Concentration
DMC	(Independent) Data Monitoring Committee
DRM	Data Review Meeting
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
FDA	Food and Drug Administration
FVIII	Coagulation factor VIII
FVIII:C	Factor VIII coagulation activity
IMP	Investigational Medicinal Product
IU	International Unit
iv	Intravenous
IVR	In vivo recovery
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean residence time
PK	Pharmacokinetic
PP	Per-protocol
PT	Preferred term
r(h)FVIII	Recombinant (human) FVIII
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
sc	Subcutaneous
SMQ	Standardised MedDRA Query
SOC	System organ class
SOP	Standard Operating Procedure
t_{max}	time for reaching maximum plasma concentration
Vd	Apparent distribution volume

VWF	Von Willebrand factor

4 General

This statistical analysis plan reflects study protocol SubQ8-01, version 7.0 dated 06-Jul-2020. It follows the principles of the Guidelines ICH Topic E3 and ICH Topic E9.

It gives all details for the final statistical analysis of all available data of enrolled patients in cohorts 1, 2, 3 and 5, except one patient who is still on immune tolerance induction (ITI) treatment. For this patient, all data reported until the time of the database snapshot on 14-Jan-2021 will be used in this analysis.

A separate statistical analysis plan will be created for the final analysis of the newly defined cohort 6.

4.1 Analyses planned and already performed

After the completion of each cohort, pharmacokinetic (PK) and safety data were analyzed and discussed during a Data Monitoring Committee (DMC) meeting. Data from each cohort were analyzed separately. This statistical analysis plan (SAP) covers the final analysis of the study as described above.

4.2 SOPs to be followed

The statistical analysis will be carried out according to Metronomia Standard Operation Procedures (SOPs).

5 Overview of the protocol

5.1 Objectives of the study

The primary objective is to assess the safety of various doses of OCTA101 (human-cl rhFVIII and recombinant von Willebrand Factor dimer) after subcutaneous (sc) injection.

The secondary objectives of this study are to assess

- the pharmacokinetics of FVIII:C after single sc injection of OCTA101
- the dose proportionality of FVIII:C after sc injections of different doses of OCTA101
- the bioavailability of sc OCTA101 compared to iv injection of Human-cl rhFVIII
- the pharmacokinetics of OCTA12 (recombinant human von Willebrand Factor fragment dimer) after single injection of OCTA101
- plasma trough and peak levels of FVIII:C after daily injections of OCTA101
- plasma trough and peak levels of OCTA12 after daily injections of OCTA101
- the efficacy of sc injections of OCTA101 in preventing bleeding episodes (BEs)

5.2 Study design

SubQ8-01 is a first-in-human, prospective, open-label Phase 1/2 study of OCTA101, a novel combination product, which consists of an eluate of an already authorized human recombinant coagulation factor FVIII product (Human-cl rhFVIII = Nuwiq®) along with OCTA12 (at a molar ratio of 1:6, with regards to FVIII binding sites in OCTA12), an investigational recombinant

human VWF fragment dimer, which is expected to increase the bioavailability of FVIII on sc dosing. The trial was planned to be carried in adult patients with severe hemophilia A according to a staged integrated design with five planned consecutive cohorts. No patient can participate in more than one cohort.

One part of the study was to be a single ascending dose study in 4 cohorts (50, 100, 200 and 400 IU/kg). The decision of going to the next higher dose or not was to be taken after each cohort by an external independent DMC after review of safety and tolerability data, FVIII:C plasma levels, and PK characteristics of FVIII:C and OCTA12. Any safety-relevant signals were also forwarded to the DMC for their review as they occurred. The reason(s) for not progressing to a higher dose level, e.g. dose-limiting toxicities (DLTs), were to be documented if such a decision was reached. Based on the results from Cohorts 1 and 2, the sponsor and DMC decided that escalation to higher doses should not take place and that further characterization of the 50 IU/kg dose was warranted. Therefore, Cohort 3 received 50 IU/kg administered into the abdomen, Cohort 4 was to receive 50 IU/kg administered into the thigh (this was not proceeded), and Cohort 5 investigated dose linearity at a lower dose range than initially planned (20, 40 and 60 IU/kg instead of 50, 100 and 200 IU/kg).

Patients in Cohort 3 also received a single intravenous (iv) dose of 50 ± 5 IU/kg Human-cl rhFVIII (Nuwiq) in order to compare the bioavailability of sc OCTA101 with those of Human-cl rhFVIII (the FVIII part of OCTA101) injected iv. The treatment sequence was be Human-cl rhFVIII followed by OCTA101.

Dose proportionality of OCTA101 was evaluated after single sc administration of three different doses (20 IU/kg, 40 IU/kg and 60 IU/kg) in 4 patients (Cohort 5).

The cohorts are summarized as follows:

- Cohort 1: 50 IU/kg (n=4): single-period investigation with a single sc dose of 50 IU/kg OCTA101 profiled up to 72 hours after dosing.
- Cohort 2: 100 IU/kg (n=4): single-period investigation with a single sc dose of 100 IU/kg OCTA101 profiled up to 96 hours after dosing.
- Cohort 3: 50 IU/kg (n=8): two-period investigation of a single iv dose of 50 IU/kg rFVIII (Human-cl rhFVIII) profiled for up to 72 hours after dosing followed by sc dose of 50 IU/kg OCTA101 administered into the abdomen profiled up to 72 hours. Treatments were to be administered in fixed sequence, with Human-cl rhFVIII first.
- Cohort 4: Cancelled.
- Cohort 5 (n=4): three-period investigation of single sc doses of 20, 40, and 60 IU/kg OCTA101 profiled up to 72 hours after dosing. Treatments were to be administered in fixed dose-ascending sequence.
- Cohort 1 and 2 PK assessment after 3-month daily dosing (n=8): single-period investigation with a single sc dose of 50 IU/kg OCTA101 profiled up to 120 hours after dosing for FVIII and up to 21 days for OCTA12.
- Cohort 6 (n=16): following an initial 4 to 6-week run-in period with Human-cl rhFVIII iv prophylaxis, 3-month daily prophylactic treatment with 12.5 IU/kg OCTA101 sc, then 25

IU/kg OCTA101 sc, and then 40 IU/kg OCTA101 sc (exact dosing depends on vial strength). The analysis of this data is not in the scope of this SAP.

By DMC-recommendation, patients enrolled in Cohorts 1, 2, and 3 proceeded to 3-month prophylactic treatment part of the study and received daily dosing using one injection (approximately 40-60 IU/kg; dose chosen to allow administration by single injection; it was permitted to daily alternate the vial size, e.g. 3000 IU on one day 1, 5000 IU on day 2, 3000 IU on day 3, 5000 IU on day 4 etc.) of OCTA101 for 3 months (home treatment). Patients from Cohort 1 received prophylactic Human-cl rhFVIII after the initial PK assessment (home treatment) until the DMC had confirmed that OCTA101 can be used as daily prophylaxis. Bleeding events during the study were to be treated with Human-cl rhFVIII. Throughout this part of the study, FVIII inhibitors, plasma concentrations of FVIII:C, and OCTA12 were measured repeatedly.

A PK assessment was performed in all patients from Cohorts 1 and one patient from Cohort 2 who completed their 3-month daily injection period for further characterizing the PK of FVIII:C and OCTA12. Blood samples were collected up to 21 days in order to see a decline in OCTA12 plasma concentrations (and allow determination of half-life) as the initial PK period was not long enough to see a decline of OCTA12. Concomitant measurement of FVIII:C up to 120 h will allow evaluation of any interaction between OCTA12 plasma concentration and FVIII:C.

Independent expert evaluation by the DMC has steered study progress for the stepwise dosage increments by cohort and the progression of patients from the first three cohorts to 3-month daily dosing. In addition, the DMC has advises on changes of the proposed dosage for the assessment of bioavailability (Cohort 3) and dose-proportionality (Cohort 5) and changes in the schedule of PK blood sampling and/or the duration of profiling without substantially increasing the total amount of blood to be sampled. Based on the observation of a second patient with inhibitors in Cohort 2, the DMC recommended placing the study on hold. the following changes were recommended for the restart of the study: cancel Cohort 4, cut Cohort 5 to 4 patients instead of the planned 8, and introduce Cohort 6.

Therefore, only the following data is available and will be analyzed:

1. Data of Cohorts 1, 2 and 3 including their 3-month daily injection period data as planned. For Cohort 3, the 3-month daily injection period was discontinued after about 4 weeks.
2. Data of Cohort 5 (planned n=8) available for 4 patients only.
3. Repeated PK assessments after 3-month daily injection period in Cohorts 1 and 2 (planned n=8) available for 5 patients (4 in Cohort 1 and one patient in Cohort 2)

5.3 Sample size

The sample size was determined based on pre-clinical findings and clinical reasoning, and was discussed with the FDA. No formal sample size calculation was performed.

5.4 Endpoints

5.4.1 Primary endpoints

The following safety endpoints are the primary endpoints in this study:

- Adverse events
- DLTs
- Thromboembolic events
- Local injection site reactions
- Inhibitor formation to FVIII

5.4.2 Secondary endpoints

Efficacy

- PK parameters of FVIII:C (see below)
- PK parameters of OCTA12 (see below)
- Total annualized bleeding rate during 3 months of daily sc treatment with OCTA101
- Spontaneous annualized bleeding rate during 3 months of daily sc treatment with OCTA101
- Total annualized treated bleeding rate during 3 months of daily sc treatment with OCTA101
- Spontaneous annualized treated bleeding rate during 3 months of daily sc treatment with OCTA101
- Traumatic annualized bleeding rate during 3 months of daily sc treatment with OCTA101
- Joint annualized bleeding rate during 3 months of daily sc treatment with OCTA101
- FVIII:C trough and peak plasma levels during 3-month daily dosing
- Score (4-point) to assess efficacy of treatment of BEs with Human-cl rhFVIII

PK parameters

- C_{max}: maximum observed concentration
- T_{max}: time of occurrence of C_{max} after dosing
- $AUC_{(0\text{-}tz)}$: quantifiable area under the time course of the concentrations de-rived by means of the combined linear/log-linear trapezoidal rule
- λ_z: apparent terminal log-linear rate constant
- $t_{½}$: apparent terminal log-linear half-life
- $AUC_{(tz\text{-}\infty)}$: AUC extrapolated beyond the last time point with quantifiable concentrations calculated as $C^*(t_z)/\lambda_z$ in which $C^*(t_z)$ is the concentration at time t_z fitted according to λ_z
- $AUC_{(0\text{-}\infty)}$: total AUC = $AUC_{(0\text{-}tz)}$+ $AUC_{(tz\text{-}\infty)}$
- $AUC_{extrapolated}$: extent of AUC extrapolation = $AUC_{(tz\text{-}\infty)}/AUC_{(0\text{-}\infty)}$
- $AUMC_{(0\text{-}tz)}$ and $AUMC_{(0\text{-}\infty)}$: quantifiable and total area under the statistical first moment curve
- MRT: mean residence time = $AUMC_{(0\text{-}\infty)}/AUC_{(0\text{-}\infty)}$
- IVR: *in vivo* recovery = dose- and body weight-normalised maximum gain in FVIII:C
- CL: total clearance (iv dosing only) = $Dose/AUC_{(0\text{-}\infty)}$
- Vd: estimated distribution volume (iv dosing only) = CL.MRT

Safety

- Antibody formation to OCTA12
- OCTA12 plasma levels during 3-month daily dosing
- Routine lab tests compared to baseline
- Vital signs compared to baseline
- Physical examination results compared to baseline

6 General Aspects of the Statistical analysis

6.1 Analysis sets

The **safety analysis set** (SAF) will include all patients who received at least one dose of IMP.

The **full analysis set** (FAS) defined according to the intention-to-treat (ITT) principle will include all patients who received at least one dose of IMP in the context of repeat daily prophylactic dose (40-60 IU/kg) for 3 months.

The **per-protocol (PP) set**, i.e., a subset of the FAS, will exclude patients with major protocol deviations which may have an impact on the evaluation of the primary study outcome parameter(s).

- Examples of major and minor protocol deviations are described in section 6.2 of this SAP.
- A final decision about the classification of protocol deviations and their consequences regarding assignment of patients to analysis sets will be made during the data review meeting. Decisions and outcomes will be approved by the Sponsor.

The **PK analysis set** will include all patients from the SAF who started the initial PK assessment with OCTA101.

The **PK–PP analysis set** will be a subset of the PK analysis set. It will exclude patients not fully evaluable for PK endpoint. Examples of criteria to exclude any patient from this analysis set are described in section 6.2 of this SAP. A final decision about the inclusion into the PK-PP analysis set will be made during the data review meeting. Decisions and outcomes will be approved by the Sponsor.

The analysis of safety will be based on the safety set by cohort.

The analysis of data from the repeat dosing will be based on the FAS as primary analysis and on the PP set as secondary analysis. This analysis will be performed by cohort and overall.

The evaluation of PK data will be based on the PK and on the PK-PP analysis sets.

6.2 Protocol deviations

The following protocol deviations are examples for major deviations with respect to the per-protocol analysis of efficacy related non-PK data. A final decision about the classification of protocol deviations and their consequences regarding assignment of patients to analysis sets will be made during the data review meeting (DRM). Decisions and outcomes will be documented in the DRM minutes, which will be approved by the Sponsor.

Table 6-1 Major protocol deviations leading to exclusion from the per protocol set

Description	Rationale
Patient used other Factor VIII product than study medication for prophylaxis of bleeding.	The analysis of annualized bleeding rates is biased if other products were used.
Patient deviated substantially from the dose of study medication foreseen in the protocol.	The analysis of annualized bleeding rates is biased if the study medication dosage did not follow the protocol defined dose.

The following protocol deviations are examples for major **deviations** with respect to the per-protocol analysis of PK data. A final decision about the inclusion into the PK-PP analysis set will be made during the data review meeting. Decisions and outcomes will be approved by the Sponsor.

Table 6-2 Major protocol deviations leading to exclusion from the PK per protocol set

Description	Rationale
Missing PK samples due to sampling issues do not allow a reliable determination of the PK parameters	Reliable estimates of the parameters are the basis for the statistical analysis

The following protocol deviations are defined as **minor**.

Table 6-3 Minor protocol deviations

Description	Rationale
Patient had a secondary diagnosis that was excluded for safety reasons. Patient could complete the study without any problems.	Does not affect the analysis of annualized BEs or other efficacy parameters
Vital signs were not measured in the scheduled time frame and vital signs are not part of the primary efficacy endpoint.	Does not affect the analysis of primary or secondary endpoints.

All protocol deviations including classification and reasons for exclusion from any analysis set will be presented in by-patient listings.

6.3 Changes or deviations from planned analyses

As the study was discontinued, fewer data are available than planned. Therefore, the following changes from planned analyses will be implemented:

All analysis based on per-protocol (PP) set will be not done as this was mainly intended for the bleeding rate analysis, and this will not be performed as originally planned.

7 Definitions for statistical analysis

7.1 Handling of withdrawals (drop-outs), missing values and outliers

If not specified otherwise, missing values will not be displayed and no imputations will be performed. Patients who dropped out from the study will be analyzed with all data provided.

In general, it is assumed that the quality of data for the 16 patients treated for 3 months on a daily basis in a single investigational site will be good and that questions related to BEs or treatment of BEs can be clarified prior to database lock.

The following rules will be applied for the analysis of BEs:

If it is unclear whether any day or site with bleeding should be counted as a separate BE due to missing or unclear data, a conservative approach will be used to count unclear dates or sites as separate BEs. In a sensitivity analysis, these date and sites will not be counted as separate BEs.

When the type of a BE cannot be determined due to missing data, the BE will be counted within the total annualized bleeding rate but not for the bleeding rates that require the type of bleeding. In a sensitivity analysis, such BEs will be counted within all possible types of bleeding.

If missing data does not allow the assessment whether a BE was successfully treated or not, the BE will be omitted from the denominator for the proportion of successfully treated BEs. In a sensitivity analysis, such a BE will be analyzed as a not successfully treated BE.

7.2 Baseline

A baseline assessment is the last assessment prior to the first injection of OCTA101. A baseline assessment may take place on Day 1, prior to the actual sc injection.

7.3 Reference day / Day 1

The day of the first treatment with study medication OCTA101 will be Day 1 for all patients for the PK part in the study, except for Cohort 3, where the first treatment with Human-cl rhFVIII within the PK part of the study will be defined as Day 1.

Patients who enter the 3-month daily prophylactic part will be assigned a second reference day, the day of first treatment with OCTA101 during the prophylactic part.

8 Statistical analysis Specification

8.1 Specifications related to whole analysis

8.1.1 Tables

If not explained differently below, summary tables will be stratified by cohort and by product/dose if applicable.

Summary tables for single dose cohorts will be presented showing the result of each cohort in the same summary table.

Summary tables related to data collected during the 3-month daily dosing period will be based on the overall FAS and will not be stratified by the previous cohort.

For continuous data, the basic statistics sample size, (number of missing values), mean, and standard deviation; minimum, first quartile, median, third quartile and maximum will be shown. Categorical data will be displayed in frequency tables showing sample size and absolute and relative frequency. The time course of continuous data will be presented using the basic statistics for each visit and the differences from baseline to last value and relative differences from baseline to last value.

8.1.2 Data listings

Electronic case report form (eCRF) and diary data will be listed as documented as well as all relevant generated and transformed variables next to the original data items. In all listings, cohort and analysis population for each patient will be included. The listings will be sorted by cohort and patient identifier.

8.2 Disposition of patients

The number and percentage of subjects screened, allocated to a cohort, received IMP, completed the full course of the trial and discontinued prematurely (incl. reasons for premature discontinuation) will be summarized by cohort and overall.

Demographic data and all other baseline characteristics and tests performed to assess the eligibility of patients will be summarized by cohort and overall for the SAF and the FAS.

8.3 Medical history and concomitant diseases

Medical history and concomitant diseases will be coded using the MedDRA dictionary in the version and the update strategy as defined in the coding guideline for this study. A concomitant disease is defined as any diagnosis, for that either no stop date was documented or the stop date is later than day 1. Incomplete stop dates will be estimated using the last possible date in order to define whether a disease is concomitant.

Summary tables presenting number and percentage of patients with medical history and with concomitant diseases by MedDRA system organ class (SOC) and MedDRA preferred term (PT) will be provided cohort and overall for the SAF and the FAS. In these tables, SOCs and PTs within SOCs will be ordered with decreasing frequency in the total column.

8.4 Previous and concomitant medication

Prior and concomitant medications excluding FVIII treatments will be coded using the WHO drug dictionary Enhanced Version, in the version and using the update strategy as specified in the coding guideline for this study. Previous medication is defined as medication that started and stopped prior to Day 1. Concomitant medication is defined as medication with at least one dose taken after Day 1. Incomplete start dates will be estimated using the first possible date, incomplete stop dates will be estimated using the last possible date in order to define whether a treatment was taken concomitantly. For missing start dates, a start prior to Day 1 will be assumed.

Summary tables presenting number and percentage of patients with previous and concomitant medication (excluding FVIII treatments) will be summarized separately for the SAF and the FAS. In these tables, the medications will be decoded on the ATC level 2 (Therapeutic Subgroup). Previous and concomitant medication will be listed together with a flag indicating whether it is classified as previous medication or concomitant medication.

8.5 Previous FVIII treatment

The type of previous FVIII treatment will be summarized overall and by indication prophylactic, on demand). Further, the preferred term of the previous FVIII treatments will be summarized.

The summaries will be stratified by cohort and will be provided for the SAF and the FAS.

8.6 Efficacy (bleeding episodes)

All reported bleedings with all details (see below) will be listed. In addition, the relative time of onset of bleeding to the OCTA101 daily injection period will be determined and listed as "before/during/after" category.

8.6.1 Data collection

For any bleeding episode (BE) occurring during the study, the following data will be recorded:

- BE type (spontaneous, traumatic, postoperative, other)
- BE site
- BE severity (minor, moderate, major, life-threatening)
- Date and time the BE first occurred or was first noticed
- Date and time the BE ended
- Dates and times the IMP (OCTA101 or Human-cl rhFVIII) was injected
- Dates and times the IMP was injected, if applicable
- IMP dose(s) and batch number(s)
- Assessment of the efficacy of treatment at the end of the BE; see below

All of these parameters will be documented by the patient (together with the investigator in case of on-site treatments) in the patient diary.

At the end of a BE, treatment efficacy will be assessed by the patient (together with the investigator in case of on-site treatment) using the following predefined criteria:

- **Excellent**: Abrupt pain relief and/or unequivocal improvement in objective signs of bleeding within approximately 8 hours after a single infusion
- **Good**: Definite pain relief and/or improvement in signs of bleeding within approximately 8–12 hours after an infusion, requiring up to 2 infusions for complete resolution
- **Moderate**: Probable or slight beneficial effect within approximately 12 hours after the first infusion, requiring more than 2 infusions for complete resolution
- **None**: No improvement within 12 hours, or worsening of symptoms, requiring more than 2 infusions for complete resolution

8.6.2 Definition of BEs

If the treatment of a BE at one site is interrupted for more than 48 hours, the events are recorded and will be analyzed as two separate BEs; if another than the original bleeding site is affected, the events are recorded and will be analyzed as separate BEs at any time.

If there are several simultaneous bleeding sites, each bleeding site is recorded and will be analyzed as a separate BE.

For certain major bleeding events, e.g., iliopsoas bleeds or bleeding into target joints, it may be necessary to continue treatment beyond the resolution of the acute phase to prevent recurrent hemorrhage. These additional treatments should be recorded in the diary (and eCRF) but will not be evaluated as treatments of the BE.

All efficacy ratings assessed as either ‘excellent’ or ‘good’ will be considered ‘successfully treated.’

8.6.3 Analysis of BEs

For the current planned analysis of cohorts 1 to 5, the analysis as described below will not be performed since the daily dosing period had to be interrupted and then fully stopped due to inhibitor formation.

All bleeding episode will be presented for each patient in the trial, with a clear flag, whether the bleeding occurred prior to, during, or after the 3-month daily dosing period with OCTA101.

All time periods of prophylactic treatment and all bleeding episodes during the 3-month daily prophylactic part will be pooled as y (number of BEs) and t (the pooled time intervals in days).

The total annualized bleeding rate is defined as $\lambda_{year} = y*t/d$, with d = 365.25 days, a standardized year.

The spontaneous annualized bleeding rate, the traumatic annualized bleeding rate, the total annualized treated bleeding rate, the spontaneous annualized treated bleeding rate, and the joint annualized bleeding rate will be calculated in the same way, by restricting the count of bleedings to the respective type of bleeding.

In addition, the prior total annualized bleeding rate of patients who entered the 3-month daily prophylactic treatment based on all bleedings reported during the 6 months prior to the first dose of OCTA101 will be calculated in the same way and presented.

All other data collected for BEs and the efficacy assessments at the end of BEs will be analyzed descriptively. The analysis will be stratified by the product that was used to treat the bleeding, if applicable.

The proportion of BEs successfully treated with Human-cl rhFVIII and (if applicable) successfully treated with OCTA101 will be presented.

8.7 Diary data

All diary data collected will be analyzed as described in the other sections of the SAP. Data from diaries will be merged together with the data from the eCRF prior to the statistical analysis.

8.8 Safety

Safety and tolerability data will first be analyzed for each cohort after the first dose(s) and evaluated by an external DMC.

All safety and tolerability parameters (adverse events, vital signs, laboratory monitoring, and physical examination results) will be listed by patient and cohort.

Analyses based on data from repeat dosing from Cohort 1, 2, and 3 with OCTA101 for 3 months will be analyzed separately from data related to the first doses. In these analyses, data will be presented by cohort and overall. The analyses will thus be based on the SAF and the FAS.

8.8.1 Study drug exposure

Study drug exposure during the PK part of the study will be analyzed by cohort and by study drug for cohort 3.

Summary tables of number of injections, total dose applied in IU and in IU/kg will be presented by cohort and PK visit.

The following data will be presented for the treatment with OCTA101 during the daily 3-month treatment period by cohort and overall:

- Duration of the OCTA101 daily treatment period in days
- Number of OCTA101 exposure days
- Number of OCTA101 injections per exposure day
- Average dose (in IU/kg) and in absolute IU per week of OCTA101
- Average dose (in IU/kg) and in absolute IU per injection of OCTA101
- Average dose (in IU/kg) and in absolute IU per injection for each visit during the daily 3-month treatment period

The number of injections per exposure day during the daily 3-month treatment period will be calculated as the number of injection sites when one vial was used and as the number of vials when more than one vial was used. Further, summary tables of total dose applied in IU and in IU/kg will be presented by cohort and overall for each visit during the daily 3-month treatment period.
All available data of study drug exposure will be listed.

8.8.2 Inhibitor and antibody formation

The frequencies (including percentages) of any inhibitor formation to FVIII and antibody formation to OCTA12 will be presented in summary table by cohort and overall and all available data will be listed.

Inhibitor formation to FVIII will be considered if at least one result of neutralizing antibody was equal or greater than 0.6 BU/ml. Antibody formation to OCTA12 will be defined as positive, if after a positive assay at screening, a confirmatory test is also positive.

8.8.3 Adverse events

Adverse events (AEs) will be coded using the MedDRA dictionary in the version and using the update strategy as specified in the coding guideline for this study.

All AEs reported during the study will be analyzed and presented. AEs starting at or after the first dose of OCTA101 (treatment emergent AE [TEAEs]) will be presented separately from those starting **prior** to the first dose of OCTA101 (pre-treatment AEs).

A TEAE related to OCTA101 is defined as any TEAE considered by the investigator to have at least a "possible" relationship with the study medication, this will also include TEAEs reported with a relationship to study medication of "Unclassified" or with a missing relationship. The relationship with the study medication will be also assessed by the Sponsor. This assessment will be listed.

The number and percentage of patients with TEAEs and the number of TEAEs will be summarized by primary MedDRA SOC and PT. Separate tables showing all TEAEs, all serious TEAEs, all non-serious TEAEs and all TEAEs related to OCTA101 will be generated. Further a summary of TEAEs by severity, presenting the most severe event per patient overall and within each SOC and PT will be generated.

Thromboembolic events and local injection site reactions will be presented within these clusters, using the summaries detailed above.

The definition of the cluster thromboembolic events will be based on the standardised MedDRA query (SMQ) “Embolic and thrombotic events”:

Definition: Thrombotic disorders are diseases characterized by formation of a thrombus that obstructs vascular blood flow locally or detaches and embolizes to occlude blood flow downstream. Embolism is the sudden blocking of a vessel by a clot or foreign material which has been brought to its site of lodgment by the blood current. (Thrombo-)phlebitis is an inflammation of a vein (phlebitis) associated with thrombus formation (thrombosis).

This SMQ includes 3 sub-SMQ:

- Embolic and thrombotic events, venous (SMQ)
- Embolic and thrombotic events, arterial (SMQ)
- Embolic and thrombotic events, vessel type unspecified and mixed arterial and venous (SMQ)

Local injection site reactions will be identified directly via the CRF entry (tickbox).

8.8.4 Local injection reactivity

Patients will evaluate injection site pain using a visual analog scale (VAS) from “0 mm/no pain” to “100 mm/extreme pain” immediately after injection completion. The local injection reactivity after single injections in the PK part will be summarized descriptively by cohort and PK visit and by overall for the follow-up PK visits.

The summaries will be provided stratified by injection site (abdomen or thigh) and by number of daily injections and overall.

Investigator (and patient in case of home treatment) shall assess local injection reactivity directly after injection and at 15 ± 5 min post-injection as described in ISO10999-10 standard:

- 0=no skin reactivity;
- 1=mild (subject is aware of the signs/symptoms, but finds it easily tolerated)
- 2=moderate (discomfort enough to cause interference with usual activities)
- 3=severe (subject is incapacitated and unable to work or participate in many or all usual activities).

For the injections during the daily 3-month treatment with OCTA101, the frequencies of local injection reactivity ratings by the investigator and the patient directly after injection and 15 ± 5 min post-injection will be summarized over the whole 3-month period using the number of injections as denominator. Visual analogue scale assessments of injection site pain will be summarized analogously.

8.8.5 Safety laboratory

Safety laboratory parameters will be analyzed using descriptive statistics for measured values at each visit and time point and for changes from pre-dose values prior to each single injection of OCTA101 for the SAF and the FAS.

8.8.6 Vital signs

Vital sign parameters will be analyzed using descriptive statistics for measured values at each visit and time point and for changes from pre-dose values prior to each single injection of OCTA101 for the SAF and the FAS.

8.8.7 Physical examination

Physical examination data will be displayed in patient data listings only.

8.9 Pharmacokinetic data

In general for the analysis, non-quantifiable pre-dose levels will be set equal to zero. In the event of quantifiable levels prior to dosing, the baseline level will be subtracted from the post-dosing values and the further analysis is focused on the post-dosing changes from baseline.

All data collected at unscheduled visits will be not used in the PK analysis.

8.9.1 Descriptive summaries for all cohorts

For each of the investigation periods after 20, 40, 60, 50 and 100 IU/kg sc of OCTA101 and for the investigation period after 50 IU/kg iv of Human-cl rhFVIII in Cohort 3, the concentrations of each analyte will be analyzed with geometric means, geometric coefficient of variation, arithmetic mean, standard deviation, minimum, maximum and median by time point (and visit) for the PK and PK-PP analysis set.

For each of the investigation periods after 20, 40, 60, 50 and 100 IU/kg sc of OCTA101 and for the investigation period after 50 IU/kg iv of Human-cl rhFVIII in Cohort 3, the PK parameters of each analyte (see section 5.4) will be analyzed with geometric means, geometric coefficient of variation, arithmetic mean, standard deviation, minimum, maximum and median for the PK and PK-PP analysis set.

Graphical displays of individual and of summarized data by cohort will support the summary tables.

In general, the dose-adjusted PK parameters will be determined using the “actual” dose in IU that was administered to a patient. The actual dose will be derived from the nominal dose and the actual potencies determined by the central laboratory for each batch separately.

The same analyses will be performed for the plasma concentrations of FVIII:C and OCTA12 samples and for the PK parameters of each analyte (see section 5.4) after the single dose administration of 50 IU/kg OCTA101 after the completion of the 3-months daily dosing period of Cohort 1 and Cohort 2.

These analyses will be reported in the relevant PK reports.

Summary tables of non-compartmental PK parameters of FVIII:C and OCTA12 will be additionally created for each cohort (cohorts 1, 2 and 3) and each period (cohort 5).
Summary tables of plasma concentrations of FVIII:C and OCTA12 will be created by cohort and overall for all visits during the 3-months daily dosing period.

8.9.2 Bioavailability analysis for Cohort 3

Single-dose data from Cohort 3 comparing the pharmacokinetics after sc and iv dosing (Human-cl rhFVIII) will be used to derive estimates of the absolute bioavailability after sc dosing and to assess route dependency of the further pharmacokinetic criteria.

Mixed effects models will be estimated for the PK-PP analysis set for each parameter of FVIII:C (dose-adjusted $AUC_{(0-\infty)}$, $AUC_{(0-tz)}$, IVR and C_{max}) with the natural logarithm of the dose-adjusted PK parameter as outcome and route (sc versus iv) as explanatory variable and a random intercept for each subject. Point and 95% confidence interval estimates of the ratio of the treatment means (sc versus iv) will be derived by back-transforming the model estimates of the difference of the treatment means on the log scale to the original scale. The SAS code will be similar to the following statements:

```
proc mixed;
class route(ref=""IV") subjid;
model logauc_adj = route;
lsmeans route / diff alpha=0.05;
random subjid;
run;
```

By means of a similar mixed effects model, estimates of the differences of the treatment means will be derived for $t_{½}$ and MRT of FVIII:C. The SAS code will be similar to the following statements:

```
proc mixed;
class route(ref=""IV") subjid;
model t12 = route;
lsmeans route / diff alpha=0.05;
random subjid;
run;
```

Point and 95% confidence interval estimates for the difference will be directly obtained from the model output. Further, summary tables of non-compartmental PK parameters used in the mixed model will be created for each analyte.

8.9.3 Dose proportionality analysis for Cohort 5

Data from Cohort 5 covering the pharmacokinetics after single sc. doses of 20, 40 and 60 IU/kg OCTA101 will be used to derive estimates of the dose-proportionality of the pharmacokinetics of FVIII:C and OCTA12.

Since the sample size for this cohort was reduced to 4 subjects, the planned statistical analysis may not be feasible due to non-convergence of the calculated model.

Analysis of C_{max} and AUC

To this purpose, the power model as described by e.g. Smith et al. (2000) will be used. A mixed effects model will be estimated for the PK-PP analysis set for each analyte and parameter (FVIII:C C_{max} and $AUC_{(0-\infty)}$ and OCTA12 C_{max}, $AUC_{(0-\infty)}$ and $AUC_{(0-tz)}$) with natural logarithm of the parameter of interest as outcome variable and the natural logarithm of the planned dose as (continuous) explanatory variable and a random intercept per subject. The SAS code will be similar to the following statements:

```
proc mixed;
class subjid;
model logauc = logdose / alpha=0.1;
random subjid;
run;
```

This corresponds to the following model equation:

$$\ln(AUC_{ij}) = (\beta_0 + \eta_{1i}) + \beta_1 * \ln(dose_{ij}) + \varepsilon_{ij}$$

where AUC_{ij} is the dose-adjusted AUC under *j*th dose (*j*=1, 2, 3 corresponding to doses 20 IU/kg sc, 40 IU/kg sc, 60 IU/kg sc of OCTA101) for the *i*th subject (*i*=1,...,4) and η_1 is the vector of subjects random intercepts.

The parameter estimate for logdose (natural logarithm of dose) $\beta_1$and its 90% confidence interval will be presented and compared to the critical region based on the following formula:

$$1 + \frac{\ln(\Theta_L)}{\ln(r)} \; ; \; 1 + \frac{\ln(\Theta_H)}{\ln(r)}$$

where Θ_L and Θ_H are set to 0.5 and 2.0 and *r* is the ratio of the highest and the lowest target dose (60 IU/kg sc / 20 IU/kg sc = 3). This yields the following critical region for β_1 and its confidence interval:

$$0.4 \; ; \; 1.6$$

Additionally, mixed effects models as specified above will be estimated for each analyte and parameter with the additional inclusion of a random slope per subject into the mixed model. The SAS code will be similar to the following statements:

```
proc mixed;
class subjid;
model logauc = logdose / alpha=0.1;
random intercept logdose / sub=subjid;
run;
```

This corresponds to the following model equation:

$$\ln(AUC_{ij}) = (\beta_0 + \eta_{1i}) + (\beta_1 + \eta_{2i}) * \ln(dose_{ij}) + \varepsilon_{ij}$$

where AUC_{ij} is the dose-adjusted AUC under *j*th dose (*j*=1, 2, 3 corresponding to doses 20 IU/kg sc, 40 IU/kg sc, 60 IU/kg sc of OCTA101) for the *i*th subject (*i*=1,...,4), η_1 is the vector of subjects random intercepts and η_2 is the vector of subjects random slopes.

The fixed parameter estimate for logdose (natural logarithm of dose) β_1 and its 90% confidence interval will be presented and compared to the above mentioned critical region, only if the variance-covariance matrix of the random effects ("G matrix") is found to be positive-definite.

Analysis of t½ and MRT

In order to check for a potential dose-dependency of t½ and MRT, mixed models with outcome t½ and MRT will be estimated for the PK-PP analysis set. The SAS code will be similar to the following statements:

```
proc mixed;
class subjid;
model t12 = dose / alpha=0.1;
random subjid;
run;
```

This corresponds to the following model equation:

$$t12_{ij} = (\beta_0 + \eta_{1i}) + \beta_1 * dose_{ij} + \varepsilon_{ij}$$

where $t12_{ij}$ is the $t_{1/2}$ under *j*th dose (*j*=1, 2, 3 corresponding to doses 20 IU/kg sc, 40 IU/kg sc, 60 IU/kg sc of OCTA101) for the *i*th subject (*i*=1,...,4) and η_1 is the vector of subjects random intercepts. Further, summary tables of non-compartmental PK parameters used in the mixed model will be created by period for each analyte.

8.9.4 Plasma trough and peak levels after daily injections of OCTA101

FVIII:C and OCTA12 trough and peak plasma levels during 3-month daily dosing period will be analyzed with geometric means, geometric coefficient of variation, arithmetic mean, standard deviation, minimum, maximum and median for the FAS. Graphical displays by visit of individual and average trough and peak levels of FVIII-C and OCTA12 will support the summary tables. Also, individual graphical displays of both analytes by time point of measurement will be provided.

This analysis will be included in the relevant PK report.

9 Software and statistical programming

The derivation of PK parameters will be performed using PCModfit v6.0.

The statistical analysis will be performed using the SAS® statistical software package, version 9.4 or higher.

SAS programming will be performed according to Metronomia standards as defined in BM-08-SOP “Statistical Analysis and Programming” and related work instructions. Special attention will be paid to planning and performance of quality control measures as documented in the QC plan for the analysis of this study (see also BM-08-WIN03 “How to Plan and Document QC for Statistical Analysis”).

10 References

ICH Topic E9: Statistical Principles for Clinical Trials5 February 1998, adopted by CPMP, March 1998, issued as CPMP/ICH/363/96

ICH Topic E3: Structure and Content of Clinical Study Reports, adopted by CPMP, December 1995, issued as CPMP/ICH/137/95

SAS Institute Inc. 2017. Base SAS® 9.4 Procedures Guide, Seventh Edition. Cary, NC: SAS Institute Inc. https://documentation.sas.com/?cdcId=pgmsascdc&cdcVersion=9.4_3.4&docsetId=pgmsashome&docsetTarget=home.htm&locale=en

Smith BP et al (2000), Confidence Interval Criteria for Assessment of Dose Proportionality, Pharmaceutical Research, Vol. 17, No. 10, pp. 1278-83