Cover Page for Statistical Analysis Plan

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Official title of study:	A multi-centre, open-label trial evaluating efficacy, safety and pharmacokinetics of nonacog beta pegol when used for treatment and prophylaxis of bleeding episodes in Chinese patients with haemophilia B
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Statistical Analysis Plan

NN7999-4670

A multi-centre, open-label trial evaluating efficacy, safety and pharmacokinetics of nonacog beta pegol when used for treatment and prophylaxis of bleeding episodes in Chinese patients with haemophilia B

Redacted statistical analysis plan
Includes redaction of personal identifiable information only.

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Version history

This Statistical Analysis Plan (SAP) for trial NN7999-4670 is based on the protocol *A multi-centre, open-label trial evaluating efficacy, safety and pharmacokinetics of nonacog beta pegol when used for treatment and prophylaxis of bleeding episodes in Chinese patients with haemophilia B*, version 1.0 dated 21Jan2021.

Table 1 SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1		Not Applicable	Original version

1 Introduction

The rationale for this phase 3b trial is to investigate the efficacy, safety and pharmacokinetics of nonacog beta pegol used for treatment of bleeding episodes and for prophylaxis in Chinese patients with moderate to severe haemophilia B (FIX activity ≤2%) in order to enable a comparison of efficacy, safety and pharmacokinetic data from the current trial in Chinese patients with data from the global pivotal phase 3a trial with nonacog beta pegol (Trial NN7999-3747).

Specifications of tables, figures and listings (TFL) and other specifications not included in this SAP will be described in the mock TFL.

1.1 Objectives and endpoints

Primary objective

- To evaluate the clinical efficacy of nonacog beta pegol in haemostasis (treatment of bleeding episodes during on-demand and prophylaxis [PPX]) in Chinese patients aged 12-70 years with moderate to severe haemophilia B

Secondary objectives

In Chinese patients aged 12-70 years with moderate to severe haemophilia B:

- To evaluate the clinical efficacy of nonacog beta pegol in PPX treatment (number of treated bleeding episodes during PPX)
- To evaluate the consumption of nonacog beta pegol
- To evaluate the immunogenicity of nonacog beta pegol
- To evaluate the general safety of nonacog beta pegol
- To evaluate the pharmacokinetic properties of nonacog beta pegol

1.2 Trial design

This trial is a multi-centre, open-label phase 3b trial evaluating the clinical efficacy, safety (including immunogenicity) and pharmacokinetics of nonacog beta pegol during on-demand and for prophylactic treatment in Chinese patients aged 12-70 years with haemophilia B and a FIX activity of ≤2%.

A total of 30 patients will be included in the trial (15 in each treatment arm) and a minimum 12 patients in each of the two treatment arms must complete the trial. The trial will have two arms: Arm A (on-demand/PPX treatment arm) and Arm B (PPX treatment arm including single-dose and

steady-state pharmacokinetic assessment). Patients will not be randomised to Arm A and Arm B. Whether a patient will be in Arm A or Arm B is the choice of the patient and the investigator and will be decided at the screening visit. The duration of the trial for each individual patient will be up to 64 weeks, i.e. 2 weeks screening period, up to 58 weeks treatment period (only applicable in Arm A in case a patient has no or few bleeds during the trial) and 30 days follow-up period.

Please refer to protocol section 4.1 for more details.

2 Statistical hypotheses

No confirmatory hypotheses are planned to be tested due to the low number of patients to be included in this trial.

3 Sample size determination

No formal sample size calculations have been performed. Based on meeting communication with CDE, a total number of 24 Chinese patients to complete this trial is assessed to be sufficient to evaluate efficacy and safety in the Chinese population, considering that data are also available from the global pivotal trial NN7999-3747, which included Asian patients. In order to account for withdrawn patients, 30 patients will be started on trial product (15 patients in Arm A [on-demand/PPX] and 15 patients in Arm B [PPX]).

All 15 patients in Arm B will participate in the pharmacokinetic evaluation to ensure that at least 12 patients will complete single-dose and steady-state pharmacokinetic profiles.

4 Analysis sets

All patients exposed to nonacog beta pegol in this trial will be included in the full analysis set (FAS) and the safety analysis set (SAS).

Exceptional outlier pharmacokinetic profiles and/or individual plasma FIX activities may be excluded when analysing pharmacokinetic endpoints based on the FAS. The rules to exclude data points from analysis of pharmacokinetic endpoints are described in this SAP, and the decision of data exclusion will be made during a review prior to database lock. Any excluded data points will be documented in the database lock minutes and also in the clinical trial report (CTR).

5 Statistical analyses

5.1 General considerations

Novo Nordisk will be responsible for the statistical analyses. The SAP will be finalised prior to first patient first visit.

Unless otherwise stated, all endpoints will be presented in total and by treatment regimen/period as applicable (on-demand period in Arm A; PPX period in Arm A; and Arm B [PPX]).

Summaries for continuous endpoints will include total number (N), mean (standard deviation), median and min/max and for pharmacokinetic endpoints also geometric mean and coefficient of variation (CV%). Summaries for categorical endpoints will include total number (N), number for each outcome category (n) and percentage for each outcome category (%).

FIX activities below lower limit of quantification (LLOQ) will be set to half the value of the LLOQ.

All bleeding endpoints will be evaluated based on bleeding episodes requiring treatment with haemostatic drugs. Non-treatment requiring bleeding episodes will only be listed. Bleeding episodes in relation to major surgery will also only be listed, i.e. not included in the analysis of bleeding endpoints.

Multiple bleeding locations occurring from the same event (e.g. due to a bicycle accident) or at the same time point will be counted as one bleeding episode and only one haemostatic response will be considered for the bleeding episode. In case different responses were registered for different bleeding locations in such a bleeding episode, the worst response will be used in the analysis.

A re-bleed is defined as a worsening of symptoms in the same location after an initial period of improvement, either on treatment or within 72 hours after stopping treatment. If a bleed occurs in the same location later than 72 hours after stopping the treatment it is considered a new bleed. Re-bleeds will only be considered for joint bleeds since the specific location for other bleeds e.g. in subcutaneous are not captured in the database.

In the sub-analyses of annualised bleeding rate (ABR) where there are less than 5 patients or no bleedings in a sub-group, the confidence interval will not be calculated.

5.2 Subject disposition

See mock TFLs.

5.3 Primary endpoint analysis

Endpoint title	Time frame	Unit	Details

Haemostatic effect of nonacog beta pegol when used for treatment of bleeding episodes during on-demand and PPX	From start of treatment (week 0) until end of treatment (up to week 50)	Count	Assessed as success/failure based on a four-point scale for haemostatic response (excellent, good, moderate and none) by counting excellent and good as 'success' and moderate and none as 'failure'

This endpoint will be evaluated based on data from both Arm A and Arm B.

The primary endpoint of haemostatic effect of nonacog beta pegol will be summarised by dichotomizing into success/failure based on counting excellent and good as 'success' and moderate and poor as 'failure', and based only on reported responses. For the calculation of success rate, the numerator will include all treated bleeding episodes with a reported haemostatic response of good or excellent, while the denominator will include all treated bleeding episodes with a reported haemostatic response. Furthermore, a sensitivity analysis will be conducted, where the denominator also includes treated bleeding episodes with missing response (i.e. missing response is counted as failure).

Haemostatic effect as measured by the four-point scale will also be summarised and listed.

The haemostatic effect will be summarised in total, by cause of bleed and by location of bleed (including haemostatic effect in target joints).

Haemostatic effect for bleeding episodes in relation to major surgery will not be included in the analysis.

5.4 Secondary endpoints analysis

5.4.1 Secondary efficacy endpoints

Endpoint title	Time frame	Unit
Number of treated bleeding episodes during PPX treatment (Arm B only)	From start of treatment (week 0) until end of treatment (week 50)	Count
Consumption of nonacog beta pegol for treatment of bleeding episodes	From start of treatment (week 0) until end of treatment (up to week 50)	IU/kg per bleed

Consumption of nonacog beta pegol for PPX treatment (Arm B only)	From start of treatment (week 0) until end of treatment (week 50)	IU/kg per year
FIX trough levels during PPX treatment (Arm B only)	From start of treatment (week 0) until end of treatment (week 50)	IU/mL

5.4.1.1 Number of treated bleeding episodes during PPX treatment

This endpoint will be evaluated based on data from Arm B only.

This endpoint will be analysed by a Poisson regression model allowing for over-dispersion (using Pearson's chi-square divided by the degrees of freedom) with the logarithm of prophylaxis duration used as offset. Estimates of ABR will be provided with 95% confidence intervals.

Only the observed bleeding episodes will be counted and the offset will be actual PPX duration.

A sensitivity analysis based on negative binomial regression model with the logarithm of PPX duration used as offset will also be performed.

Another sensitivity analysis will be performed imputing the number of bleeding episodes for withdrawals. Specifically this will be done by using a last observation carried forward (LOCF) approach for all patients with at least 1 month PPX treatment duration by calculating the yearly bleeding episode rate for withdrawn patients and use that as endpoint. As an example a patient with 6 bleeding episodes in 4 months will have an endpoint value of 18 corresponding to a maintained bleeding rate of 18 bleeding episodes per year. For patients withdrawing within 1 month, this method is considered to give too uncertain LOCF values, hence imputation will not be attempted for such patients.

ABRs will be summarised in total, by cause of bleed, and by location of bleed (including ABR in target joints).

Bleeding episodes in relation to major surgery will not be included in the analysis.

ABR will also be performed separately for patients coming from an on-demand regimen and a PPX regimen. For both groups the ABR will be compared descriptively to the ABR immediately prior to initiation of PPX with nonacog beta pegol.

5.4.1.2 Consumption of nonacog beta pegol for treatment of bleeding episodes

This endpoint will be evaluated based on data from both Arm A and Arm B.

The number of injections per bleed will be summarised and listed.

The number of IU/Kg per bleed will be summarised and listed.

5.4.1.3 Consumption of nonacog beta pegol for PPX treatment

This endpoint will be evaluated based on data from Arm B only.

The number of injections for PPX treatment (i.e. excluding treatment of breakthrough bleeds) will be summarised and listed.

The number of IU/Kg per year will be summarised and listed.

5.4.1.4 FIX trough levels during PPX treatment

This endpoint will be evaluated based on data from Arm B only.

The FIX trough and recovery levels will be analysed by a mixed model on the log-transformed plasma FIX activity with patient as a random effect. The mean trough and recovery levels will be presented back-transformed to the natural scale together with the 95% confidence interval.

The following rules will be implemented for the analysis of trough and recovery levels:

- Pre-dose and post-dose values will be excluded if post-dose activity is ≤ pre-dose activity
- Pre-dose and post-dose values taken less than 5 days or more than 9 days after last dose will be excluded
- Pre-dose and post-dose values taken less than twice the PPX treatment interval (14 days) after last treatment of a bleeding episode will be excluded
- Pre-dose and post-dose values taken within 2 weeks after surgery will be excluded
- Pre-dose and post-dose values taken in relation to the first exposure will be excluded
- FIX activity measured in defrosted plasma samples will be excluded

Furthermore, trough and recovery (30 minutes post-dose) levels measured at scheduled visits will be summarised and listed. Incremental recovery will be calculated and presented in summary tables and plots.

5.4.2 Secondary safety endpoints

Endpoint title	Time frame	Unit
Number of patients with inhibitory antibodies against FIX defined as titre ≥0.6 Bethesda units (BU)	From start of treatment (week 0) until end of treatment (up to week 50)	Count

Endpoint title	Time frame	Unit
Number of adverse events (AEs)	From start of treatment (week 0) until end of treatment (up to week 50)	Count
Number of serious adverse events (SAEs)	From start of treatment (week 0) until end of treatment (up to week 50)	Count

The safety endpoints will be evaluated based on data from both Arm A and Arm B.

5.4.2.1 Number of patients with inhibitory antibodies against FIX defined as Titre ≥0.6 BU

The rate of neutralising antibodies will be reported and a 1-sided 97.5% upper confidence limit will be provided based on an exact calculation for a binomial distribution. For the calculation of the inhibitor rate the nominator will include all patients with neutralising antibodies while the denominator will include all patients with a minimum of 10 exposures plus any patients with less than 10 exposures but with neutralising inhibitors.

5.4.2.2 AEs and SAEs reported during the trial

Treatment emergent AEs (TEAEs defined as AEs occurring after dosing with trial product) and treatment emergent SAEs (TESAEs) will be summarised by frequency of events and frequency of patients with any event. Similar summaries cross-classified by severity and by causal relation to trial product will be made.

Furthermore, listings will be provided displaying all TEAEs and TESAEs including pertinent clinical information.

5.4.3 Secondary pharmacokinetic endpoints

Pharmacokinetic parameters to be derived in the trial (Arm B only) are listed in **Table 2.**

Table 2 Definition and calculation of pharmacokinetic parameters

Title	Time frame	Unit	Details
Incremental recovery (IR)	Single-dose: 30±10 minutes post injection at week 0 Steady-state: 30±10 minutes post injection at week 12	(IU/mL)/(IU/kg)	The increase in plasma FIX activity from pre-dose to 30 min post-dose divided by the dose injected. IR is calculated by subtracting the FIX activity (IU/mL) measured in plasma at time 0 from that measured at time 30 min after dosing and dividing this difference by the dose injected at time 0 expressed as IU/kg body weight.
FIX activity 30 min post-injection (C_{30min})	Single-dose: 30±10 minutes post injection at week 0 Steady-state: 30±10 minutes post injection at week 12	IU/mL	The FIX activity recorded 30 min after end of injection. Expected to be the maximum FIX activity observed.
FIX activity 168 h post-injection (C_{168h})	Single-dose: 168±24 hours post-injection at week 0 Steady-state: 168±24 hours post-injection at week 12	IU/mL	The FIX activity recorded 168 hours after end of injection. Expected to be the minimum FIX activity observed.
$AUC_{(0-inf)}$	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	h·IU/mL	Area under the plasma activity versus time profile from time zero to infinity. Measure of total plasma exposure. $AUC_{(0-inf)} = AUC_{(0-t)} + C_{(t)} / \lambda_z$, where $C_{(t)}$ is the last measurable activity.
$AUC_{(0-t)}$	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	h·IU/mL	Area under the plasma activity versus time profile from time zero to the last measurable activity. Measure of plasma exposure in the time interval 0 to last measurable activity. $AUC_{(0-t)}$ is calculated using the linear trapezoidal method from time 0 to the time for the last measurable activity. The activity at time 0 will be estimated by log-linear back extrapolation of the two initial post-administration activities. If the second value is not lower than the first value, the concentration at time 0 will be defined as the highest of these two.

Title	Time frame	Unit	Details
$AUC_{(0\text{-}168h)}$	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	h·IU/mL	$AUC_{(0\text{-}168h)}$ is calculated using the linear trapezoidal method from time 0 to 168 hours. If FIX activities are not available up to 168 hours post-dose, the area under the missing terminal part of the curve will be determined by interpolation or extrapolation using a similar principle as for $AUC_{(0\text{-}inf)}$, where the terminal elimination rate constant, λ_z, is used given that there are sufficient measurements for the determination of the terminal elimination rate constant.
Accumulation ratio	0-168 hours post-injection at weeks 0 and 12		Accumulation ratio is calculated as $AUC_{(0\text{-}168h)}$ at steady state/$AUC_{(0\text{-}168h)}$ after a single dose.
Terminal half-life ($t_{½}$)	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	h	$t_{½} = \ln(2) / \lambda_z$, where λ_z is the terminal elimination rate constant. The terminal elimination rate constant will be estimated using linear regression on the terminal part of the log(activity) versus time profile.
Clearance (CL)	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	mL/h/kg	CL= Dose / AUC. Using $AUC_{(0\text{-}inf)}$ for single-dose and $AUC_{(0\text{-}168h)}$ for steady-state.
V_z	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	mL/kg	Apparent volume of distribution based on the terminal phase. $V_z = CL/\lambda_z$
V_{ss}	0-168 hours post-injection at week 12	mL/kg	Apparent volume of distribution at steady state. $V_{ss} = CL \cdot MRT$
%extrap	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	%	Percentage of $AUC_{(0\text{-}inf)}$ determined by extrapolation. $AUC_{(t\text{-}inf)}/AUC_{(0\text{-}inf)}$

Title	Time frame	Unit	Details
MRT	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	h	Mean residence time. MRT = $AUMC/AUC_{(0\text{-}inf)}$, where AUMC is the area under the first moment curve, i.e. the area under the curve $t \cdot C_{(t)}$, calculated with the same method as $AUC_{(0\text{-}inf)}$ (linear trapezoidal method + extrapolated area).
λ_z	Single-dose: 0-168 hours post-injection at week 0 Steady-state: 0-168 hours post-injection at week 12	1/h	Terminal elimination rate constant. The terminal elimination rate constant will be estimated using linear regression on the terminal part of the log(activity) versus time profile.

Single-dose pharmacokinetics will be based on the pharmacokinetic session at Visit 2, and steady-state pharmacokinetics will be based on the pharmacokinetic session at Visit 5.

The pharmacokinetic parameters will be calculated using plasma FIX activity obtained from one-stage clotting assay. The pharmacokinetic parameters will be derived according to a non-compartmental method, as described in Table 2. The actual time points will be used in the calculations.

If any profiles and/or individual plasma FIX activities are excluded from the primary pharmacokinetic analysis, a sensitivity pharmacokinetic analysis will also be performed and reported based on all observed data. The primary pharmacokinetic analysis is based on the FAS excluding outliers.

Which specific pharmacokinetic data points and profiles that will be excluded will be defined prior to database lock. Profiles with pre-dose FIX activity >5% at Visit 2 (possibly indicating inadequate wash out) and profiles that are not indicative of a normal i.v. injection (i.e. clearly increasing plasma FIX activity initially) should be excluded. If a patient is treated with an additional dose during the pharmacokinetic session, the plasma concentrations after the occurrence will then be excluded. Furthermore, if the pharmacokinetic profile shows indications of an additional dose (e.g. clearly increased plasma FIX activity), the plasma concentrations after the occurrence will then also be excluded.

Individual and mean pharmacokinetic profiles will be presented graphically.

Furthermore, the mean pharmacokinetic endpoints will be summarised and individual pharmacokinetic endpoints will be listed.

5.5 Exploratory endpoints analysis

Not applicable.

5.6 Other safety analyses

All additional safety parameters such as laboratory parameters, vital signs and physical examinations will be summarised and listed.

5.7 Other analyses

Surgery

In relation to surgery the following will be recorded:

- Haemostatic effect evaluated on the four-point scale (excellent, good, moderate and none) and assessed by the investigator/surgeon on the day of surgery (Day 1) and on the last day in the post-operative period the patient is at the trial/surgery site.

- Loss of blood and requirements for transfusion on the day of surgery (Day 1) and during the post-operative period Days 2-7 or until the last day the patient is at the trial/surgery site, whatever comes first.

All records will be summarised and listed. The date of return to regular trial treatment needs to be recorded, to allow for the exclusion of major surgery period from exposure time and the calculation of ABR and haemostatic response.

5.8 Interim analyses

No interim analysis is planned.

5.8.1 Data monitoring committee

Refer to protocol Section 9.6

6 Supporting documentation

6.1 Appendix 1 List of abbreviations

ABR	Annualised bleeding rate
AE	Adverse event
AUC	Area under the curve
AUMC	Area under the first moment curve
BU	Bethesda unit
CDE	Centre of Drug Evaluation
CI	Confidence interval
CL	Clearance
CTR	Clinical trial report
CV%	Coefficient of variation in percent
FAS	Full analysis set
FIX	Coagulation factor nine
IR	Incremental recovery
LLOQ	Lower limit of quantification
LOCF	Last observation carried forward
MRT	Mean residence time
PPX	Prophylaxis
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
t½	Terminal half life

TE Treatment emergent

TFL Tables, figures and listings

6.2 Appendix 3: Definition and calculation of endpoints, assessments and derivations

Not applicable

7 References

Not applicable