Cover Page for Statistical analysis plan

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Official title of study:	A multi-national trial evaluating safety and efficacy, including pharmacokinetics, of NNC 0129-0000-1003 when administered for treatment and prophylaxis of bleeding in patients with haemophilia A.
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Turoctocog alfa pegol
NN7088-3859 Ext 2 Final
Clinical Trial Report
Appendix 16.1.9

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Novo Nordisk

16.1.9 Documentation of statistical methods

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

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Statistical Analysis Plan

Trial ID: NN7088-3859

A Multi-national Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC 0129-0000-1003 when Administered for Treatment and Prophylaxis of Bleeding in Patients with Haemophilia A

Author:

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List of abbreviations

AE	*Adverse event*
ANOVA	*Analysis of variance*
AUC	*Area under the curve*
BU	*Bethesda unit*
BW	*Body weight*
C_{30min}	*FVIII activity at 30 min post injection*
CI	*Confidence interval*
CL	*Total clearance*
CTR	*Clinical trial report*
ED	*Exposure days*
EOM	*End of Main part of the trial*
FVIII	*Coagulation factor eight*
LOCF	*Last observation carried forward*
N8-GP	*Glycopegylated recombinant coagulation factor VIII (NCC 0129-0000-1003)*
OD	*Over-dispersion*
PD	*Pharmacodynamics*
PK	*Pharmacokinetics*
PPX	*Prophylaxis*
PRO	*Patient reported outcome*
SAE	*Serious adverse event*
SAP	*Statistical analysis plan*
$t^{1/2}$	*Terminal half life*
Vss	*Volume of distribution at steady state*
Vz	*Apparent volume of distribution*

1 Introduction

1.1 Trial information

This phase 3 trial is a multi-centre, multi-national, open-label, non-randomised trial evaluating safety, pharmacokinetics and clinical efficacy of NNC 0129-0000-1003 (hereafter referred to as N8-GP) when used for treatment of bleeding episodes and for long-term prophylaxis. The trial consists of a Main Phase followed by an Extension Phase part 1 and part 2. In the main phase, patients in the prophylaxis arm were receiving one single bolus dose of 50 U/kg body weight (BW) of N8-GP every 4 days (96 hours interval).

In the extension part 1, patients with a low bleeding rate on every 4 day prophylaxis in the main phase have the possibility of being randomised to either 50 U/kg BW every 4th day or to 75 U/kg BW once weekly dosing. If the patient for the last 6 months (180 days) prior to Visit 13/14 only had 0 - 2 bleeding episodes, the patient was eligible for randomisation. Patients who were not eligible to be in the randomised arm or patients who are eligible but unwilling to be randomised can stay on the same regimen as in the Main phase.

In the extension phase part 2, patients with a low bleeding rate (0-2 bleeding episodes during the last 6 months) on every 4 day prophylaxis have the possibility of changing to 75 U/kg BW once weekly dosing regimen. Patients who has been on 75 U/kg BW once weekly dosing during extension phase part 1 can continue on this regimen arm or change back to either 50 U/kg BW every 4th day if the patients want to. Patients on once weekly dosing who have more than 2 spontaneous bleeds within a 8 week period or are hospitalised due to a bleed need to change back to every 4 days regimen according to the protocol.

1.2 Scope of the statistical analysis plan

The scope of this SAP is to clarify the planed analysis for extension phase part 2 described in the protocol.

This SAP is based on the Protocol *A Multi-national Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC 0129-0000-1003 when Administered for Treatment and Prophylaxis of Bleeding in Patients with Haemophilia A* version 1.0 (30-Sep-2011) including Final Substantial Amendment no 1 version 1.0, NL (24-Oct-2011), Final Substantial Amendment no 2 version 1.0, Global (09-Dec-2011), Final Substantial Amendment no 5 version 1.0 FR (3-Feb-2012), Final Substantial Amendment no 6 version 1.0, Global (30-Mar-2012), Final Substantial Amendment no 8 RU (21-Jun-2012), Final Substantial Amendment no 10 version 2.0, Global (03-Dec-2012), Final Substantial Amendment no 11 version 1.0 IL (05-Dec-2012),Final Substantial Amendment no 12

version 1.0, Global (05-Mar-2013), Final Substantial Amendment no 13 version 1.0, Global (03-May-2013), Final Substantial Amendment no 14 version 1.0, Global (12-Jul-2013), Final Substantial Amendment no 15 version 1.0, Global (28-Aug-2013), Final Substantial Amendment no 18 version 1.0, Global (29-Nov-2013), Final Substantial Amendment no 19 version 2.0, Global (29-Apr-2016), and the final version of the protocol for this trial (version 13, 24 January 2017).

2 Statistical considerations

The Pivotal part of the trial was reported based on all data from the Main Phase where all patients have reached at least 50 EDs (except for patients having had surgery as part of pathfinder™3), and all patients have had their first visit after 50 EDs where all planned assessments including inhibitors have been performed. All main conclusions from the trial are based on this reporting except for conclusions regarding every 7 day prophylaxis and long term safety/efficacy.

In the interim at end of extension phase part 1 all data from main phase up to the end of extension phase part 1 was analysed and reported. The main focus was on once weekly prophylaxis with every 4 day dosing as control where patients with low bleeding rate could be randomised to the two treatment arms.

The main reason for the interim for the ongoing extension phase part 2 including all data from the main phase and extension phase up to the cut-off is to collect additional safety and efficacy data.

2.1   Sample Size Calculation

The study has two co-primary endpoints for the Pivotal part that both need to succeed for the study to succeed. The co-primary endpoints can reasonably be considered approximately independent and combined power then becomes the product of the individual power for each co-primary endpoint. For further detail, see section 18.1 in the protocol.

2.2   Statistical Methods

2.2.1   General Considerations

Novo Nordisk A/S will be responsible for the statistical analysis. All tests will be performed as 1-sided tests on 2.5% significance level. All bleeding endpoints will be evaluated based on bleeding episodes requiring treatment with N8-GP. Non-treatment requiring bleeding episodes that coincide with regular prophylaxis doses are not included in the analyses.

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. As stated in the protocol, 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 where there are less than 5 patients in a sub-group, model based estimation will not be done. If no bleedings occur in a sub-group, then the confidence interval will not be calculated.

2.2.2 Primary Endpoint(s)

The study has two co-primary endpoints that both have to succeed.

Incidence Rate of FVIII-inhibitors ≥0.6 BU

The rate of inhibitors 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 50 exposures plus any patients with less than 50 exposures but with neutralising inhibitors. Adequate safety with regard to neutralising inhibitors will be concluded if the upper 1-sided 97.5% confidence limit is below 6.8% roughly corresponding to the upper 97.5% confidence limit if 2 inhibitors out of 105 patients are observed (3 or less if the study should get 127 or more patients with 50 exposure days).

Annualised bleeding rate (total number of bleeding episodes per patient assessed as annualised bleeding rate) in the prophylaxis arm

A review of historical data has found that haemophilia A patients on on-demand treatment on average bleed 24 times per year while they bleed 6.8 times per year when treated on prophylaxis.

Historical Control for Hypothesis Test for Prophylaxis

Based on the studies referenced below, Novo Nordisk suggests that representative numbers for mean annual bleeding rate (ABR) in severe haemophilia patients is 24 bleeds/year for patients treated on-demand and 6.8 for patients on prophylactic treatment.

Novo Nordisk has based the estimate of historical annual bleeding rate on a systematic review of the treatment of Haemophilia A and B and von Willebrand disease, performed by the Swedish Council on Health Technology Assessment.

The referenced report is based on review of 3710 abstracts in total, covering treatment of Haemophilia A, B or von Willebrand disease. Articles were then selected for full review according to specific criteria such as administration of recombinant or plasma-derived Factor concentrate, outcome (eg number of bleeding episodes, inhibitor development or quality-of-life measurements), and number of patients included. For haemophilia A, only studies comprising 20 patients or more were selected. The main objectives of the SBU review is to evaluate the short- and long-term effects

with different treatment strategies with coagulation factor concentrates in Haemophilia A and B and von Willebrand disease.

In the search, SBU identified 37 references dealing with "replacement therapy with factor concentrates in treating severe, moderate, and mild haemophilia A". All these references identified by SBU were reviewed as full length articles at Novo Nordisk, and only the original papers with a mean total ABR reported (or at least possible to calculate) in patients with an endogenous FVIII:C activity <2% were included in our analysis.

Table 2–1   Historical mean total annual bleeding rate in Haemophilia A patients with an endogenous FVIII:C activity <2%

SBU Ref No	Reference	On-demand (mean ABR)	Number of patients	Prophylaxis (mean ABR)	Number of patients
4	*Courter, 2001*[3]	*18*	*27*	*4*	*27*
5	Lusher, 2003[4]	23	85	10	85
5	*Lusher, 2003*[4]	*N.R.*	*N.R*	*6*	*45*
9	Tarantino, 2004[5]	N.R.	N.R.	6.3	107
10	Nemes, 2007[6]	N.R.	N.R.	4.9	49
11	Recht, 2009[7]	N.R.	N.R.	3.9	94
15	Abshire, 2000[2]	34	38	8.3	27
17	Smith, 2005*[8]	N.R.	N.R.	10.3	32
18	*Manco-Johnson, 2007*[1]	*18*	*33*	*3.3*	*32*
25	Schwartz, 1990**[9]	19	56	N.R.	N.R.
	Weighted Mean	24	179	6.8	394

ABR = Annual Bleeding Rate. N.R. = not reported. Studies in *italics have* not included adults. No = Reference number in SBU report, chapter 3.1.1. * 5/32 are moderate Hemophilia A, **4/56 are moderate Hemophilia A

In the studies, the mean ABR in patients on prophylaxis is consistently lower than in patients treated on-demand, as expected. The studies have ABR in the range of 18 – 34 and 3.3 – 10, for on-demand and prophylaxis treatment, respectively, and include different numbers of subjects. To arrive at a representative ABR number from the various studies, we weighted the estimates from each study based on the number of subjects included.

As the proposed trial with N8-GP will only include patients aged 12 years and above, we further suggest to exclude 3 of the 9 historical studies[1,3,4], that only include children below age 6, in order to better reflect the study population. The historical mean ABR for on-demand and prophylaxis in haemophilia A with <2% will then be estimated at 24 and 6.8, respectively. However, if also the

studies including children below 6 years of age are included, the estimated ABR for on-demand and prophylaxis are 23 and 6.4, respectively. The 2% cut off is often used in clinical studies and will give a conservative estimate of the ABR in the study population in the proposed trial with a cut off of <1%.

Prophylactic effect of N8-GP will be concluded if the bleeding rate is significantly below 50% of the historical on-demand bleeding rate (i.e. significantly lower than 12) as well as within 25% of the historical prophylaxis bleeding rates (i.e. significantly lower than 6.8*1.25 = 8.5). Since both must be met in practice it must be shown that the bleeding rate is significantly lower than 8.5.

Let AR be the true yearly bleeding rate. The null-hypothesis will be tested against the alternative hypothesis as given by:

H0: $AR \geq 8.5$ against HA: $AR < 8.5$

The endpoint will be analysed by a Poisson regression model on number of bleeding episodes per patient allowing for over-dispersion (using Pearson's chi-square divided by the degrees of freedom (i.e. Scale=Pscale in SAS)) and using log observation duration as an offset. Estimates of the annualised bleeding rates will be provided with 95% confidence intervals.

For patients withdrawing prematurely, the number of bleeding episodes counting in the analysis will be imputed up to what they could be expected to have had if they had completed the trial. If e.g. a patient withdraws after 2 months with 3 bleeding episodes, but the patient should have been in the study for 12 months, then this patient will in the analysis count as having had 18 bleeding episodes in 12 months. This is similar to last observation carried forward (LOCF) and will avoid positive bias occurring from patients with many bleeding episodes withdrawing early.

The rules for calculating planned exposure time used for imputation depends on what part of the trial the patient is withdrawn from and furthermore what parts of the trial the calculations represent as described in the following.

Calculations of ABR for the Pivotal part:
Since the planned exposure time for each patient in the Pivotal phase of the trial depends on when the patient was recruited in the trial, the maximum expected exposure time based on the last visit date included in each part of the analysis will be used as planned exposure time, e.g. the date of the last patient's first visit after his 50EDs will be used for the pivotal part of the analysis where all patients should have reached at least 50 EDs (except for patients having had surgery as part of pathfinder™3).

Calculations of ABR for extension phase part 1:

For patients who were withdrawn in the Pivotal part the planned exposure time will be until the end of the pivotal part as described above. For patients who were withdrawn in the main part after the pivotal part ended, the maximum expected exposure time will be used, i.e. 100 weeks as this is the maximum duration for main phase. For patients withdrawn during extension phase part 1, the planned exposure is 24 weeks (duration of extension phase part 1) added to the actual exposure in main

For calculations only including the randomised period, the planned exposure time will be the time from visit 14 to visit 17, i.e. 24 weeks. Patients that change from Q7D to non-randomised part of Q4D will have their bleeds imputed using a planned exposure time of 24 weeks using the same approach as in the pivotal part of the trial even though they are not withdrawn from the study. This is to avoid bias for randomized Q7D caused by patients withdrawing due to high bleeding rate.

Calculations of ABR for extension phase part 2:
For patients withdrawn prior to extension phase part 2, planned exposure is calculated as explained above. For patients withdrawing during extension phase part 2, planned exposure in extension phase part 2 will be calculated using the overall cut-date (15Aug2017) as planned end date. The actual exposure time in main and extension phase part 1 is added to the planned exposure time in extension phase part 2, to get the overall planned exposure time for a withdrawn patient.

Planned exposure time is only relevant for the treatment regimen the patient is on, when being withdrawn. Time on other regimens and time in surgery trial is subtracted when calculating planned exposure time.

For patients who withdrew within 1 month, imputation will be conducted by assuming an annualised bleeding rate of 24 (bleeds per year) for the missing period. This also applies to change from Q7D to Q4D in the randomised part of extension phase part 1.

Estimates of the annualised bleeding rates for each regimen will be provided together with the 95% confidence intervals as described in the protocol.

Sensitivity analyses:
Analysis applying a different model
A sensitivity analysis based on a negative binomial regression model with number of bleeding episodes requiring treatment as the outcome variable, and adjusting for exposure time will be performed.

Analysis without imputation to planned trial duration

The primary prophylaxis analysis will be repeated but without imputing number of bleeding episodes for any withdrawals. Instead only the observed bleeding episodes will be counted and the offset will be actual observation duration rather than planned.

Analysis imputing by LOCF without imputation for withdrawals occurring within the first month

The primary analysis will be repeated but without using imputation of 24 bleeding episodes for withdrawals within the first month. Instead only the observed bleeds and the observed duration will be used for such subjects.

Analysis imputing a minimum of 24 bleeds per year to planned trial duration

A conservative sensitivity analysis will be performed imputing for all withdrawals the observed bleeding rate into the missing period if it is greater than 24 per year and otherwise 24 per year. As an example a patient with 3 bleeds in 2 months (observed ABR 18) that should have been in the study for 12 months will have 20 bleeds imputed for the missing 10 months. Patients with 6 bleeds in 2 months (observed ABR 36) will have 30 bleeds imputed into the missing 10 months.

Analysis of 12 months data from patients that could have had 12 months prophylaxis

Since patients will stay in the Main Phase of the trial until the same end of Main Phase date some patients will get only about 7 months prophylactic treatment while others may get more than 19 months (except for patients entering the trial with the intention of major surgery after the end of normal recruitment period). To investigate if the varying durations have any impact on the results a sensitivity analysis will be performed looking only at 12 months data from patients with planned trial duration of 12 or more months. Otherwise this analysis will be performed similarly to the primary analysis. This sensitivity analysis is not applicable after the interim for extension phase, part 1, since no new data for the main phase and extension phase part 1 will be available.

To investigate the effect over time the bleeding rates per month will be calculated and summarised numerically and graphically. This will be done by treatment regimen and only including the first consecutive period on each treatment regimen. A patient that is on different treatment regimens during the trial will therefore have bleeding rate per month calculated for each treatment regimen, but only including the first consecutive period on each treatment regimen. Surgery leave is not considered change in treatment regimen.

Similarly the bleeding rate by calendar month will be calculated and presented. If there is a calendar effect it is conceivable that it would be reversed in the southern hemisphere compared to the

northern. Therefore, calendar months for Australia and Brazil are shifted 6 months, i.e. January is changed to July, February to August etc.

2.2.3   Confirmatory Secondary Endpoints

Haemostatic effect of N8-GP when used for treatment of bleeding episodes, assessed 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.

In order to protect against false significances this endpoint will only be analysed as a confirmatory secondary endpoint in the pivotal part if the analyses of the co-primary endpoints are both successful. Otherwise this endpoint will be analysed only as a supportive secondary endpoint.

This is assessed as success/failure based on a four-point scale for haemostatic response (excellent, good, moderate and none). Excellent and good counts as success and moderate and none as failure. In addition any bleeding episodes with missing response information will be counted as failures.

A success rate of 80% is considered the goal. Due to variation it is not certain that N8-GP will achieve an observed 80% success rate in this trial even if the true success rate is 80%. For that reason it will be demonstrated that the success rate for N8-GP is at most 15% (absolute) worse than 80%.

Let R be the true success rate. The null-hypothesis will be tested against the alternative hypothesis as given by:

H0: R ≤ 65% against HA: R > 65%

Specifically this will be done by a logistic regression. The analysis will be performed by use of Proc Genmod in SAS. Correlation within patients will be taken into account using a Generalized Estimation Equations approach with a working correlation matrix with a compound symmetry structure. Adequate efficacy will be concluded if the 1-sided lower 97.5% confidence limit for the success rate is above 65%.

Response as measured by the four point scale will also be summarised and listed. Bleeding episodes will come both from the on-demand periods and from prophylaxis periods. This will be analysed together in the statistical analysis but will also be summarised by treatment regimen.

Sensitivity analyses

Analysis on observed responses only (i.e. excluding missing observations)
A sensitivity analysis will be performed similar to the primary analysis but only analysing bleeding episodes with recorded responses (i.e. not counting any bleeding episodes with missing response as failures).

Analysis imputing missing haemostatic response based on number of infusions used
A sensitivity analysis will be performed using the number of infusions given to impute the haemostatic response for missing data. Specifically missing responses where only 1 dose was used will be counted as success while missing responses where 2 doses were used will be counted as failures.

Analysis imputing missing haemostatic response based on patients recorded responses on other bleeding episodes
A sensitivity analysis will be performed where missing treatment responses will be imputed based on recorded treatment responses from that particular patients. The imputation will be done randomly based on the recorded success rate. As an example if a patient has 2 missing responses and 4 treatment successes in 5 treated bleeds with recorded response then the missing responses will be imputed randomly based on an 80% success chance. Patients with missing responses for all treated bleeding episodes will be imputed as failures for all episodes.

2.2.4 Supportive Secondary Endpoints

Consumption of N8-GP (number of infusions and U/Kg) per bleed
The number of infusions per bleed will be summarised and listed.
The number of U/Kg per bleed will be summarised and listed.

Consumption of N8-GP (number of infusions and U/Kg per month and per year) during prophylaxis and on-demand treatment
The number of infusions per month and per year will be summarised and listed by treatment regimen (prophylaxis and on-demand).

The number of U/Kg per month and per year will be summarised and listed by treatment regimen (prophylaxis and on-demand).

Haemostatic effect as measured by recovery and trough levels FVIII:C (in all patients in the prophylaxis treatment arm)
Recovery and trough levels measured at scheduled visits will be summarised and listed.

2.2.4.1 PK Endpoints

- FVIII activity 30 min post-injection (C_{30min})
- Incremental recovery, ([IU/mL] / [U/kg])(single dose and steady state)
- Trough level, (IU/mL) (single dose and steady state)
- Area under the curve (AUC), (h*IU/mL)

- Terminal half-life ($t_{\frac{1}{2}}$), (h)
- Clearance (mL/h/kg)
- Mean Residence time (MRT) (h)
- Volume of distribution at steady state (V_{ss}) (mL)
- Apparent volume of distribution(V_z) (mL)
- Percentage of $AUC_{(0\text{-}inf)}$ determined by extrapolation (%extrap)
- Terminal elimination rate constant (λ_z)

Single dose PK will be based on the subgroup of patients with full PK sessions at Visits 2 and 7. Steady state PK will be based on the periodic recovery and pre-dose measurements on all patients from Visits 3 to 13:

Single dose PK
The PK parameters will be calculated using plasma concentration obtained from chromogenic assay and clot assay. The PK parameters will be derived according to a non-compartmental method, as described in Table 2–2. The actual time points will be used in the calculations.
If any profiles and/or individual plasma concentrations 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 full analysis set excluding outliers.
Specifically which data points and profiles that will be excluded will be defined prior to database lock. It should exclude profiles with pre-dosing activity > 5% (possibly indicating inadequate washout) and profiles that are not indicative of a normal intravenously short infusion administration (e.g. clearly increasing plasma concentrations initially). If a patient is treated with an additional dose during the PK session, the plasma concentrations after the occurrence will then be excluded. Furthermore, if the profile shows indications of an additional dose (e.g. clearly increased plasma concentration), the plasma concentrations after the occurrence will then also be excluded.
All pharmacokinetic endpoints (except MRT, Vss, Vz, percentage of $AUC_{(0\text{-}inf)}$ determined by extrapolation and Terminal elimination rate constant) will be analysed using mixed effects model on log-transformed parameters including visit as fixed effect, and patient as random effects. Estimates of each endpoint with 95% confidence intervals will be provided back-transformed to the natural scale. In addition, AUC and C_{30min} will be compared between the 2 visits as estimated ratio between the two visits together with the 90% confidence intervals.
All PK profiles will be presented graphically by subject and by visit.
Furthermore, the mean PK endpoints will be summarised and individual PK endpoints will be listed.

Table 2–2   Definition and Calculation of PK Parameters

Parameter	Description	Calculation
Incremental Recovery	Dose-normalised activity recorded 30 min after end of infusion and reported as [IU/mL]/[U/kg]. Expected to be the highest dose-normalised activity observed.	The incremental recovery is calculated by subtracting the FVIII 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 U/kg BW
C_{30min}	The FVIII activity recorded 30 min after end of infusion. Measure of maximum exposure	The FVIII activity recorded 30 min after end of infusion. The value is sensitive to deviations from the scheduled sampling time. This limits the value of this parameter, especially when comparing to other studies.
Trough level	Activity recorded immediately before next dose is given and reported as [IU/mL]. Expected to be the lowest dose-normalised activity observed.	
$t_{1/2}$	Terminal half-life	$t_{1/2} = \ln(2) / \lambda_z$, where λ_z is the terminal elimination rate. The terminal elimination rate will be estimated using linear regression on the terminal part of the log(activity) versus time profile
CL	Total plasma clearance of drug after intravenous administration	CL= Dose / AUC
$AUC_{(0-inf)}$	Area under the activity versus time profile from time zero to infinity. Measure of total plasma exposure.	$AUC = AUC_{(0-t)} + C_{(t)} / \lambda_z$, where $C_{(t)}$ is the last measurable activity.
$AUC_{(0-t)}$	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.
V_z	Apparent volume of distribution based on the terminal phase	$Vz = CL/\lambda z$
V_{ss}	Apparent volume of distribution at steady-state	$Vss = CL \times MRT$
%extrap	Percentage of AUC(0-inf) determined by extrapolation	AUCt-inf/(AUC(0-inf))
MRT	Mean Residence Time	MRT = AUMC/AUC(0-inf), where AUMC is the area under the first moment curve, i.e. the area under the curve t·C(t), calculated with the same method as AUC(0-inf) (linear trapezoidal method + extrapolated area)
λ_z	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

Since no new single dose PK profiles have been collected since main phase, PK parameters will not be reported for the extension phase part 2 interim.

Steady state PK

The following rules will be implemented for pre-dose activity, post dose activity and incremental recovery:

- FVIII activity data will be excluded if post-dose activity is ≤ pre-dose FVIII activity.

- Samples drawn more than ±2 days from planned time as well as less than twice the prophylaxis treatment interval after last treatment of bleed will be excluded.

- For patients performing a PK session at Visit 7 (including a wash-out period), the pre- and post-dose data will be excluded from calculation of mean trough and mean recovery. The incremental recovery will still be calculated.

- Samples for randomised patients in extension phase part 1 changing from every 7 day dosing to every 4 day dosing will be excluded from change of treatment regimen and onwards.

- Samples taken at "return visit" from the surgery trial (NN7088-3860)

- FVIII activity measured in defrosted plasma samples will be excluded.

The pre-dose FVIII activity levels assessed from after at least 4 PPX dosing intervals will be analysed to get an estimate of the pre-dose level for N8-GP at steady state. The model will be a mixed model on the logarithmic plasma activity levels with dose and age as a factor and patient as a random effect. Plasma concentrations below lower limit of quantification (LLOQ) will be set to half the value of the LLOQ. The estimated pre-dose FVIII level will be presented together with the 95% confidence intervals. Trial duration for the main phase differs between patients, but all patients reach at least Visit 8 prior to being transferred to Visit 13 (end of main phase). Therefore, the analysis will be performed both on pre-dose values from main and extension phase part 1 and furthermore including only pre-dose values up to visit 8.

A sensitivity analysis including all data after at least 4 PPX dosing intervals will also be performed for both cases.

Furthermore the same analyses will be performed for measurements from the randomised patients in the extension phase part 1.

Pre-dose activity, post-dose activity and incremental recovery will be summarised by visit. Furthermore, mean pre-dose activity and mean post-dose activity will be plotted vs. time.

In extension phase part 2, the only PK endpoint that will be summarised by visit is incremental recovery for all prophylaxis patients, since incremental recovery is independent of dose and dosing frequency. Incremental recovery will be calculated using the same activity measurements as used for estimation of recovery and trough levels.

In extension phase part 2 the following rules have been added to the rules for activity measurements listed above (the new rules are also applied to activity measurements from main and extension phase part 1)

- Samples from visits when changing treatment regimen (e.g. from every 4 day dosing to every 7 day dosing) will be excluded (this rule replaces the rule for randomised patients changing from every 7 day dosing to every 4 day dosing)

- Samples taken at "return visit" from the PK-trial (NN7088-4033) are excluded.

- If the FVIII activity is above 3.0 IU/ml based on one of the three clot assays and the ratio of Clot PSS (chrom)/Chrom PSS > 1.5, all clot data for this sample time point will be excluded. The reason for this rule is that plasma samples can be activated during pre-

analytical handling resulting in very high FVIII activity measured with one-stage clot assay but not with the chromogenic assay.

2.3   Safety Endpoints

Adverse Events (AEs) and Serious Adverse Events (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.

Changes in vital signs (blood pressures, pulse, temperature, respiratory rate)
These endpoints will be presented and listed

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

2.4   Exploratory endpoints

Incidence of anti-N8-GP antibodies (non-neutralising)
Anti-N8-GP antibodies (non-neutralising) will be listed.

Anti-CHO HCP antibodies
Number of samples with negative/positive will be summarised by visit.

Anti-PEG antibodies
Plots will be created of incremental recovery according to PEG antibody status at baseline and during the trial.

2.5   Interim Analysis

All data from the Main Phase of the trial will be analysed and reported when all patients have reached at least 50 EDs (except for patients having had surgery as part of pathfinder™3), and all patients have had their first visit after 50 EDs where all planned assessments including inhibitors have been performed. The analysis of the main phase of the trial will be based on all data up to the patient's last visit at this point. All main conclusions from the trial will be based on this reporting.

Furthermore, an interim analysis was performed when all patients had completed extension phase part 1. The interim for the extension phase part 2 will cover all data from the main phase and extension phase 1+2 up to last visit before 15-Aug-2017.

2.6 Sequential Safety Analysis/Safety Monitoring

Not applicable (NA).

2.7 Explorative Statistical Analysis for Pharmacogenetics and Biomarkers

NA.

2.8 PK and/or PD Modelling

The relationship between plasma concentration and bleeding frequency will be investigated. Based on each individuals PK assessments (full PK from some patients but only recovery and troughs for most patients) the time to 5%, 3% and 1% FVIII activity after each dose given will be calculated and the bleeding rates for each period (>5%, 3-5%, 1-3% and <1%) will be calculated and listed and summarised.

A statistical comparison will be made between the bleeding rates above and below 1% FVIII activity. This will be done based on a Poisson regression with patient above or below 1% activity as fixed effects. The model will allow for over-dispersion (estimated using Pearsons chi-square divided by the degrees of freedom) and will use log duration (above 1% activity and below 1% activity respectively) as offset. For computational reasons only patients with at least one bleeding episode will be included and similarly only patients with at least 2 weeks cumulative time under 1% activity and 2 weeks cumulative time over 1% activity will be included.

The following PK parameters will be estimated using a one-compartment population PK model:

- CL_{70kg} (L/h)   Population median clearance in a patient of weight 70 kg
- V_{70kg} (L)   Population median volume of distribution in a patient of weight 70 kg
- $\beta_{CL,BW}$ Allometric exponent for relation between median CL and BW
- $\beta_{V,BW}$ Allometric exponent for relation between median V and BW

Extrapolated population PK parameters:

- $C_{max,SS}$ Predicted maximal plasma level of FVIII activity in steady state
- $AUC\tau$ Predicted total exposure to FVIII activity during a single dosing interval in steady state

PK and possible PK/PD modelling are considered exploratory analyses that may not be reported in the clinical trial report. The PK assay results to be used for modelling are chromogenic FVIII activities measured against N8-GP reference material.

Population PK modelling will be done as a combined analysis with data from the first human dose trial pathfinder™1 with the main scope of assessing the influence of demographic covariates such as BW and possibly race on PK properties of N8-GP. The population PK model of which maximum-likelihood parameter values are to be estimated is a linear one-compartment model parameterised with clearance CL and volume of distribution V.

The final output of the PK modelling is predictions of FVIII activity exposure parameters (C_{max} and $AUC\tau$) at steady state in proposed prophylaxis dosing regimes for patients of BW 10 kg, 25 kg, 50 kg, 70 kg and 100 kg and of any race sufficiently well represented in the trial to allow for assessment (min. 20 PK samples from min. 5 different patients).

2.9   Health Economics and/or Patient Reported Outcome

PROs will in the Main Phase be assessed through PRO questionnaires at screening visit (Visit 1) and end of trial visit for patients in the on-demand arm and for patients in the prophylaxis arm. The main PRO endpoint will be subgroup total scores. Changes in scores over time of the main PRO endpoints at Visit 1 to end of main visit will be explored and presented graphically. Evaluations of PRO data will alone be based on descriptive statistics, i.e. summary tables, listings and figures.

HE calculations will be performed separately by the Novo Nordisk HE department. Novo Nordisk A/S will be responsible for the statistical analysis.

2.10   Analyses of Extension Phase Data

Data from part 1 of the Extension Phase
The main objective of part 1 of the Extension Phase is to investigate the safety and efficacy of every 7 day dosing by evaluating ABR for this dosing regimen and comparing it to the same historical bleeding episode rates as used in the primary analysis for the co-primary efficacy endpoint in the Main Phase. Prophylactic effect of every 7 day dosing will be concluded if the bleeding rate is significantly below 50% of the historical on-demand bleeding rate (i.e. significantly lower than 12) as well as within 25% of the historical prophylaxis bleeding rates (i.e. significantly lower than 6.8*1.25 = 8.5). Since both must be met in practice it must be shown that the bleeding rate is significantly lower than 8.5.

An analysis similar to that for the co-primary endpoint in the Main Phase will be carried out for the bleeding episodes in the two randomised treatment regimens during the Extension Phase part 1, adding treatment regimen as a factor to the model. Subjects switching from the every 7 day dosing arm to the non-randomised every 4 day dosing arm will be handled as withdrawals in this analysis.

The imputation for withdrawals will be similar to that for the Main Phase, except that planned maximum duration is now 6 months.

Estimates of the annualised bleeding rates for each randomised regimen will be provided with 95% confidence intervals. Prophylactic effect of every 7 day dosing will be concluded if the upper limit of the 95% confidence intervals (CI) is below 8.5. In addition, the two randomised treatment regimens will be compared by reporting the estimated ratio between the two randomised treatment regimens with corresponding 95% confidence interval.

As for the co-primary endpoint in the Main Phase, a sensitivity analysis will be carried out where bleeding episodes for withdrawals are not imputed.

ABR based on accumulated number of bleeding episodes (Main Phase + Extension Phase part 1) will be evaluated for the every 4 day dosing regimens combined, i.e., combining the randomised and non-randomised arm. The model used will be the same as for the primary analysis of the Main Phase data.

Only treatment requiring bleeding episodes will be considered in the above evaluations of ABR Incidence rate of FVIII-inhibitor and haemostatic effect of N8-GP when used for treatment of bleeding episodes will be evaluated based on accumulated data (Main Phase + Extension Phase part 1).

Adverse events, consumption of N8-GP, recovery and pre-dose levels will be summarised based on accumulated data (Main + Extension Phase part 1) by treatment regimen combining the every 4 day dosing regimens. In addition, separate summaries including data from part 1 only will be presented by treatment regimen for the randomised subset of patients to support the main objective of part 1of the Extension Phase.

Other safety endpoints will be summarised based on accumulated data (Main Phase + Extension Phase part 1). Binding non-inhibitory antibodies will be listed.

PRO endpoints at end the Extension Phase part 1 will be evaluated and presented as described in section 18.9.

Data from part 2 of the Extension Phase

The main objective of extension phase part 2 is to collect long term safety and efficacy data.

ABR will be calculated as for the co-primary endpoint in the main phase, but based on the accumulated data, i.e. combined data from main phase, extension phase part 1 and extension phase part 2. Furthermore, sensitivity analyses are performed based on actual bleeding episodes and exposure times (i.e. without imputation for withdrawn patients).

Incidence rate of FVIII-inhibitor and haemostatic effect of N8-GP when used for treatment of bleeding episodes will be evaluated based on accumulated data.

Adverse events and consumption of N8-GP will be summarised based on accumulated data by treatment regimen.

Incremental recovery will be summarised by visit for extension phase part 2 and furthermore be presented graphically in the following ways:

- By N8-GP production process (extension phase part 2 data)
- According to Anti-PEG-antibody status (accumulated data)

For Anti-CHO HCP-antibodies, the number of negative/positive samples will be summarised by visit.

Other safety endpoints will be listed and/or plotted based on accumulated data.

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PRO endpoints for extension phase part 2 will be summarised by time since first dose (since timing of visits depend on treatment regimen). Time grouping will span a year, i.e. grouping being "1 - <2 years", "2 - <3 years",…. The two prophylaxis regimens will be considered as a combined prophylaxis treatment regimen.

2.11   Additional Evaluations

Exploratory analysis comparing results from study NN7008-3543, NN7008-3545, NN7008-3568 and NN7088-3776 with results from this study will be performed and reported separately. This analysis will include overall comparisons as well as individual comparisons for patients participating in one of the above listed trials and this NN7088-3859 trial, if the patient through the informed consent has given approval of such comparison of data.

3   Changes to the statistical analyses planned in the protocol

It has been clarified that treatment requiring bleeding episodes occurring in multiple locations with the same start time will be calculated as one bleed. This is in accordance to the initial setup of how data could be captured in the diary and electronic case report form (eCRF), but some changes have been made in the eCRF by mistake at a later time point where auto-query has been added to enquire all bleeding episodes which were associated with multiple locations to be captured as separate episodes.

As a conservative approach, the worst response will be used in case multiple haemostatic responses were registered for multiple bleeding locations with the same start time.

None of the non-joint bleeds will be considered as re-bleeds even if they occurred within 72 hours as the specific location is not captured in the database.

A conservative approach will be used to calculate the planned exposure time for withdrawals such that the maximum expected exposure time will be used.

An additional sensitivity analysis is added for the analysis of the ABR to investigate the robustness of the assumption that the treatment requiring bleeds can be modelled adequately using a Poisson distribution with overdispersion. The additional sensitivity analyses uses modelling based on the negative binomial distribution

It was added that ABR will be analysed when there are at least 5 patients in total and when at least one bleed was observed, in order to provide reliable estimates.

At the end of trial:

Efficacy data:

As a sensitivity analysis, analysis without imputation to planned duration will be performed. The other sensitivity analyses performed in the pivotal reporting are no longer applicable, since this was already addressed in the previous interim reportings.

Calculation of ABR by month for a given treatment regimen has been defined to be based on the first consecutive period on the treatment regimen to ensure that a effect over time is not influenced by time on other treatment regimens.

Calculation of ABR by calendar month has been defined to be done by shifting calendar month half a year for countries in the southern hemisphere (i.e. Brazil and Australia). This is done to take the difference in seasons between the southern and northern hemisphere into account.

The ABRs defined to be calculated based on a sub-selection consisting of data from extension phase part 2, will not be done, since patients have differing trial durations when entering extension phase part 2 and therefore ABRs for such a sub-selection is of limited value.

PK data:

Since patients are allowed to change treatment regimen at any time during the extension phase part 2 and since the visits intervals are different for the prophylaxis treatment regimens, FVIII activity data will be summarised by visit only for incremental recovery. Since incremental recovery is dose-normalised activity increase this will not be affected by a possible regimen and dose change.

Plots of pre-dose activity measurements will not be created since the plots have been evaluated to contribute with no relevant information.

PRO data:

PRO endpoints for extension phase part 2 will be summarised by grouping of time since first dose, since timing of visits depend on treatment regimen and since patients are moving between treatment regimens.

Safety data:

Adverse Events and Serious Adverse Events will be summarised by treatment regimen and all other safety endpoints will be presented by listings and individual graphical representation only. Safety data consists of accumulated data from the trial.

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