2 Synopsis

Trial Registration ID-number NCT00837356	
Title of Trial A multi-centre, multi-national, open-label sequential trial comparing pharmacokinetics and safety of turoctocog alfa[a] and Advate® in subjects with haemophilia A [a] *The trial product, turoctocog alfa, was previously named N8. The name was changed after finalisation of the protocol for this trial.*	
Investigators There was one principal investigator for each of the trial sites. Prof. [redacted] was assigned signatory investigator for the trial.	
Trial Sites Patients were enrolled in the trial at 6 sites in 4 countries; 3 European countries (Germany (sites [redacted] and [redacted]), Italy (sites [redacted] and [redacted]) and Switzerland (site [redacted])), and Israel (site [redacted]). In addition, one site in Spain was initiated but did not enrol any patients.	
Publications Martinowitz U, Bjerre J, Brand B, Klamroth R, Misgav M, Morfini M, Santagostino E, Tiede A, Viuff D. Bioequivalence between two serum-free recombinant factor VIII preparations (N8 and ADVATE®) – an open-label, sequential dosing pharmacokinetic study in patients with severe haemophilia A. *Haemophilia,* 2011; 17(6): 854-859	
Trial Period 05 March 2009 to 01 October 2009	**Development Phase** 1
Objectives *Primary Objective:* to evaluate and compare the pharmacokinetic profiles of intravenously (i.v.) administered recombinant FVIII (turoctocog alfa) and i.v. administered Advate® in non-bleeding haemophilia A patients without inhibitors. *Secondary Objective*: to evaluate the safety of turoctocog alfa in non-bleeding haemophilia A patients without inhibitors	
Methodology This was a multi-centre, multi-national, open-label, first human dose, pharmacokinetic, safety, single dose trial using a sequential design in patients with haemophilia A (Factor VIII). The trial consisted of 4 visits. After a screening visit (Visit 1), each patient had two pharmacokinetic sessions (dose visits) where the patient was dosed and dose-related blood samples and data were collected (Visits 2 and 3). Upon completion of Visit 3 (Day 3), an End of Trial Visit (Day 4) was performed. Patients received Advate® at a dose of 50 IU/kg body weight (bw) in the first session (Visit 2), and subsequently (following a washout period of 4 days after first trial product administration) turoctocog alfa at a dose of 50 IU/kg bw (second session – Visit 3). The duration of trial participation for each patient was approximately 4 weeks. The total duration of the trial was estimated to be 6 months. At each dose visit blood samples for safety parameters and pharmacokinetic analysis were drawn prior to dose and at intervals up until 48 hours post trial product administration. Pharmacokinetic assessments were based on FVIII activity determined using a one-stage clotting assay (clotting assay) and a chromogenic substrate assay (chromogenic assay). Furthermore, physical examination, vital signs, electrocardiogram, injection site inspection, and recording of adverse events were to be performed for safety assessments prior to dosing and at various time points up to 48 hours post dosing. Blood samples for inhibitor titre were collected at the screening visit (Visit 1), prior to dosing of turoctocog alfa (Visit 3), and at end-of trial (at Visit 4). In case of development of FVIII inhibitors, the patient was to attend additional follow-up visit(s) up until 3 months.	

Number of Subjects Planned and Analysed

A total of 27 patients were planned to be screened in order to allow for 23 patients to be dosed.

It was expected that at least 21 of the 23 dosed patients would complete the trial. Screening procedures were performed in a total of 25 patients; 2 patients were screening failures leaving 23 patients eligible for inclusion.

All 23 patients received trial product (both turoctocog alfa and Advate®) and hence constitute both the safety and the full analysis set.

Diagnosis and Main Criteria for Inclusion

Adult or adolescent (12 years to 55 years) previously treated patients (PTPs - 150 EDs or more) with severe (FVIII activity ≤1%) haemophilia A and without inhibitor were eligible for inclusion.

Test Product, Dose and Mode of Administration, Batch Number

Turoctocog alfa was provided by Novo Nordisk as a sterile, freeze-dried powder in a 2-8 °C stable formulation in single use vials of 2000 IU/vial (batch no. VR40187, potency: 1944 IU/vial) to be reconstituted with 4.3 mL of 0.9% sodium chloride (NaCl) (batch no. 10409642) for injection. The in-use time of the reconstituted turoctocog alfa was 24 hours at 2-8 °C or 4 hours at 9-25 °C. After reconstitution with the appropriate volume, each vial contained 500 IU/mL. The reconstituted solution was a colourless and clear/almost clear solution with pH 6.9. Turoctocog alfa was to be injected as a slow bolus i.v. in semi-recumbent position using an injection pump (5 mL/min). In Israel turoctocog alfa was administered using a slow i.v. bolus infusion without the use of pumps.

Duration of Treatment

A single dose of Advate® and turoctocog alfa was administered, separated by a wash-out period of at least 4 days.

Reference Therapy, Dose and Mode of Administration, Batch Number

Advate® was provided by Novo Nordisk as a kit including sterile, freeze-dried 1500 IU/vial (batch nos. LE01H506AJ: potency 1622 IU/vial, LE01H541AC: potency: 1505 IU/vial, LE01H519AM: potency: 1503 IU/vial) and solvent for injection (sterile water for injection). Advate® was to be reconstituted with 5 mL of sterile water for injection. After reconstitution with the appropriate volume, each vial contained 300 IU/mL Advate®.

The reconstituted solution was a colourless and clear/almost clear solution with pH 6.7-7.3.

Advate® was to be injected as a slow bolus i.v. in semi-recumbent position using an injection pump (7.7 mL/min). In Israel Advate® was administered using a slow i.v. bolus infusion without the use of pumps.

Criteria for Evaluation – Efficacy

Pharmacokinetic assessments based on FVIII activity determined using a one-stage clotting assay and a chromogenic substrate assay.

Criteria for Evaluation – Safety

- Adverse events including serious adverse events and medical events of special interest (MESIs)
- Vital signs (blood pressure, heart rate, temperature, and respiratory rate)
- ECG
- Physical examination
- Clinical laboratory assessments
- Injection site tolerability
- Development of FVIII inhibitors

Statistical Methods

- Primary pharmacokinetic endpoints: Incremental recovery, $t_{½}$, AUC and CL
- Secondary pharmacokinetic endpoints: AUC_{last}, V_{ss}, V_z, C_0, C_{max} and MRT

- The pharmacokinetic parameters were based on FVIII activity measurements. In the calculations of pharmacokinetic parameters, the measurements were regarded as concentrations. The actual sampling times were used in the calculation of the pharmacokinetic parameters using standard non-compartmental methods.

The FVIII activities were normalised to the per protocol dose of 50 IU/kg (i.e., adjusted for actual dose administered and the actual product strengths (IU/vial)) prior to calculation of the pharmacokinetic parameters and outliers were excluded from the analyses.

- The main evaluation of the pharmacokinetic endpoints was based on descriptive statistics (i.e. summary tables, listings and figures).

In addition, the primary endpoints were compared between Advate® and turoctocog alfa using a multiplicative (i.e., endpoints were log-transformed before analysis) linear mixed effects model, with treatment as a fixed effect and patient as a random effect. The treatment ratio was estimated from the model and presented together with a 90% confidence interval (CI). If the 90% CI for incremental recovery, $t_{½}$, AUC and CL is contained within the bioequivalence interval, 0.8-1.25, bioequivalence between the two treatments could be concluded.
The secondary pharmacokinetic endpoints were analysed in the same way as the primary endpoints, but MRT was not log-transformed. The treatment ratio was estimated from the model and presented together with a 90% CI. If the 90% CI for AUC_{last} and C_{max} is contained within the bioequivalence interval, 0.8-1.25, bioequivalence between the two treatments could be concluded.
- Evaluations of safety data were based on descriptive statistics.

Demography of Trial Population

All patients had severe haemophilia A, were male, and the mean age was 22 years (range: [redacted] years). Two patients were below the age of 18 years (one [redacted]-year-old and one [redacted]-year-old). The median height was 1.75 m (range [redacted] m), and the median weight was 73 kg ranging from [redacted] kg. The BMI ranged from [redacted] kg/m^2 with a median of approximately 23 kg/m^2.
Information related to the underlying gene defect [redacted]. [redacted] relatives with haemophilia A [redacted]. Clinical suspicion of inhibitors against factor VIII was not reported in any of the patients.

Efficacy Results

FVIII activity was measured using two different assays: a clotting assay and a chromogenic assay. Separate analyses of pharmacokinetic data were made on the full analysis set using unadjusted data, on dose-adjusted data and on data adjusted for dose and excluding outliers. When corrected for actual dose and strength of trial products (i.e., IU/vial) the median administered dose of Advate® was 54.0 IU/kg (mean: 53.1 IU/kg, range: 50.2-57.3 IU/kg) and the median dose of turoctocog alfa was 45.2 IU/kg (mean: 45.8 IU/kg, range: 42.5-50.0 IU/kg). As a consequence of this difference between trial products in actual dose administered, the main analyses is based on FVIII activities normalised to the per protocol dose of 50 IU/kg prior to calculation of the pharmacokinetic parameters. All data presented below are based on the analyses of data from the full analysis set adjusted for dose and excluding outliers.

Clotting Assay

- Post-dosing of turoctocog alfa the maximal plasma FVIII activity (C_{max}) was recorded within the first half-hour for the majority of patients with a mean value of 1.07 IU/mL. The mean incremental recovery of turoctocog alfa was 0.020 (IU/mL)/(IU/kg). After the end of the turoctocog alfa infusion, the FVIII activity declined in an exponential way with a tendency to a bi-exponential decay pattern. The mean V_{ss} of turoctocog alfa was 3826 mL indicating limited distribution into the extravascular space. During the terminal phase, the rate of decline in FVIII activity (i.e. the $t_{½}$) was 10.83±4.95 hours. The mean total exposure, expressed as AUC, of turoctocog alfa was 14.22±3.75 h×IU/mL. The mean residual level at 48 hours post-dosing was 0.03 IU/mL ranging from 0.0125 IU/mL (LLoQ) to 0.13 IU/mL.
- The pharmacokinetic profile of a single dose of i.v. administered turoctocog alfa was found to be similar to an i.v. administered Advate® in non-bleeding haemophilia A patients without inhibitors (see Figure 1).
- Turoctocog alfa and Advate® were found to be bioequivalent, as evaluated by incremental recovery, $t_{½}$, AUC, AUC_{last}, CL and C_{max} (see Table 1).

Chromogenic Assay

- Post-dosing of turoctocog alfa the maximal plasma FVIII activity (C_{max}) was recorded within the first half-hour for the majority of patients with a mean value of 1.54 IU/mL. The mean incremental recovery of turoctocog alfa was 0.028 (IU/mL)/(IU/kg). After the end of the turoctocog alfa infusion, the FVIII activity declined in an exponential way with a tendency to a bi-exponential decay pattern. The mean V_{ss} of turoctocog alfa was 3149 mL, indicating limited distribution into the extravascular space. During the terminal phase, the rate of decline in FVIII activity (i.e. the $t_{½}$) was 11.96±9.28 hours. The mean total exposure expressed as AUC of turoctocog alfa was 18.70±5.08 h×IU/mL. The mean residual level at 48 hours post-dosing was 0.05 IU/mL ranging from 0.0125 IU/mL (LLoQ) to 0.15 IU/mL.
- The pharmacokinetic profile of a single dose of i.v. administered turoctocog alfa was found to be similar to an i.v. administered Advate® in non-bleeding haemophilia A patients without inhibitors, although the level of FVIII

activity was slightly higher for turoctocog alfa compared to Advate® (see Figure 1).

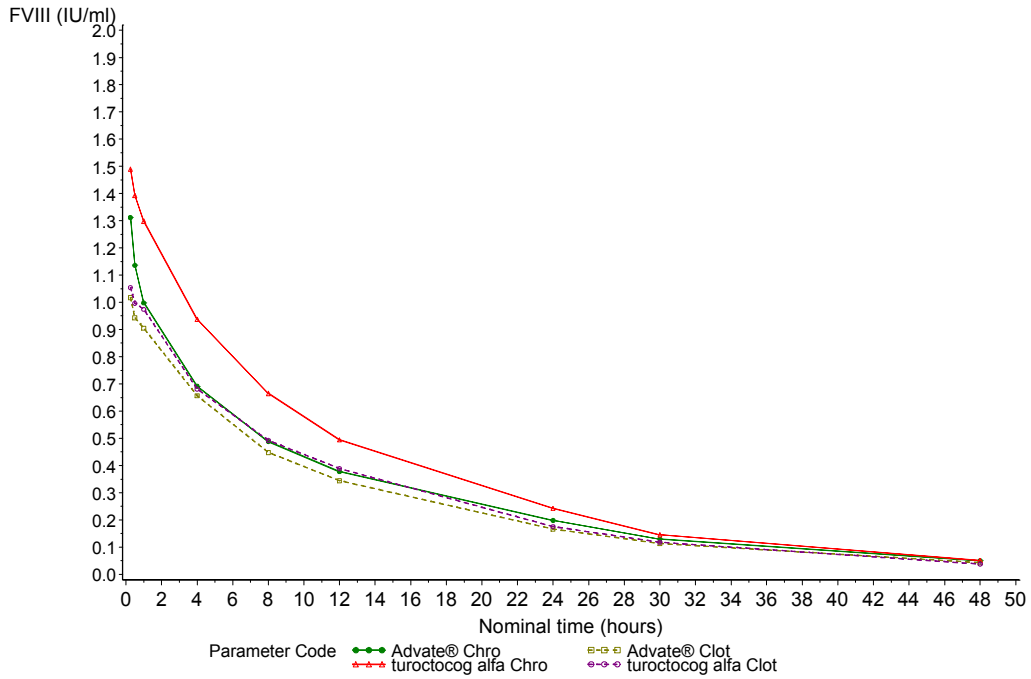
- Bioequivalence between turoctocog alfa and Advate® could not be demonstrated for any of the pharmacokinetic parameters (see Table 2).

Table 1: Clotting Assay - Pharmacokinetic Analysis Adjusted for Dose and Excluding Outliers

	Turoctocog alfa Geo. Mean (N=20)	Advate® Geo Mean (N=20)	Ratio	90 % CI	P-value
AUC	13.19	12.09	0.917	[0.860 ; 0.978]	0.031
Incremental recovery	0.020	0.018	0.921	[0.859 ; 0.987]	0.054
$t_{½}$	9.86	10.55	1.070	[0.981 ; 1.168]	0.195
Total CL	272	298	1.094	[1.022 ; 1.170]	0.033

Table 2: Chromogenic Assay - Pharmacokinetic Analysis Adjusted for Dose and Excluding Outliers

	Turoctocog alfa Geo. Mean (N=20)	Advate® Geo. Mean (N=20)	Ratio	90 % CI	P-value
AUC	18.064	14.44	0.800	[0.765 ; 0.836]	0.000
Incremental recovery	0.027	0.022	0.824	[0.773 ; 0.878]	0.000
$t_{½}$	10.34	11.45	1.107	[0.937 ; 1.308]	0.303
Total CL	199	250	1.258	[1.202 ; 1.316]	0.000



Clot: Clotting assay; Chro: Chromogenic assay; values below LLoQ are set to LLoQ/2;
Subjects [redacted], [redacted], [redacted] and [redacted] have been excluded

Figure 1: Mean Profiles of FVIII Activity versus Time for Turoctocog Alfa and Advate® – One-stage Clotting Assay and Chromogenic Assay - Full Analysis Set Adjusted for Dose and Excluding Outliers

Safety Results

- Overall, turoctocog alfa was well tolerated and the safety profile of turoctocog alfa appeared similar to that of Advate®.
- No serious adverse events were reported during the trial and no patients withdrew due to adverse events. The frequency of adverse events following administration of turoctocog alfa was low and the type and rate of adverse events were as expected.
- No formation of inhibitors against FVIII was reported in the trial.
- There were no clinically relevant changes in safety laboratory parameters, vital signs or ECG findings post turoctocog alfa dosing evaluated as related to turoctocog alfa as judged by the Investigator.
- The frequency of injection site reactions post dosing of turoctocog alfa and Advate® was similar.

Conclusions

- In this prospective comparative pharmacokinetic trial in non-bleeding previously treated patients with severe haemophilia A, the pharmacokinetic profiles of turoctocog alfa and Advate® were comparable.
- Bioequivalence between turoctocog alfa and Advate® was demonstrated based on the clotting assay, whereas pharmacokinetic parameters for turoctocog alfa and Advate® were slightly different when obtained with the chromogenic assay.
- Turoctocog alfa appeared safe and was well tolerated.

The trial was conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice (May-1996).

The results presented reflect data available in the clinical database as of 23 September 2011.