2 Synopsis

Trial Registration ID-number	**EudraCT number**
- NN1731-1668	2005-000891-42

Title of Trial

A randomised, open-label, multi-centre trial investigating the intra-subject variability of ROTEM and TEG parameters following two intravenous administrations of the same dose of activated recombinant factor VII (rFVIIa/NovoSeven) in haemophilia subjects in a non-bleeding state

Leading Principal Investigators

A principal investigator was appointed at each of the 5 trial sites. [redacted], M.D, [redacted] was appointed as the signatory investigator.

Trial Sites

1 site in Israel, 1 site in France, 1 site in Denmark (no subjects enrolled) and 2 sites in Germany.

Publications

None

Trial Period	**Development Phase**
09 November 2005 to 06 April 2006	Phase 1

Objectives

Primary Objective:

- To evaluate the intra-subject variability (*in vivo* reproducibility) of thromboelastographic parameters as measured by ROTEM® and by TEG® prior to and at 15, 60, 120 and 240 minutes following two administrations of the same dose of activated recombinant human factor VII (rFVIIa) in haemophilia subjects in a non-bleeding state

Secondary Objectives:

To evaluate whether there is a correlation between different doses of rFVIIa and the ROTEM® and TEG® parameters

- To evaluate whether there is a correlation between thromboelastographic parameters as determined by ROTEM® and by TEG®
- To compare the *in vivo* reproducibility of thromboelastographic parameters as determined by ROTEM® and by TEG®
- To evaluate the inter-subject variability of thromboelastographic parameters as measured by ROTEM® and by TEG®
- To evaluate whether there is a correlation between the response of *in vivo* and *ex vivo* supplementation of rFVIIa
- To evaluate whether there is a correlation between the *in vivo* ROTEM® or TEG® parameters and different laboratory parameters
- To evaluate the safety of rFVIIa administration.

Methodology

This clinical trial was a randomised, multi-centre, multi-national, open label, phase 1 trial to investigate the intra-subject variability of ROTEM® and TEG® parameters prior to and following two intravenous (i.v.) administrations of the same dose of rFVIIa. At the screening (visit 1), eligible subjects were randomised to receive two single administrations of 45 µg/kg, 90 µg/kg, or 180 µg/kg of rFVIIa. Subsequent to randomisation, subjects attended a trial visit on two separate occasions (visits 2 and 3), separated by at least one week, but less than 12 weeks apart. The first dosing (visit 2) could be no longer than 1 month from randomization (visit 1).Visits 1 and 2 could be performed on the same day to avoid assessment redundancy. At each visit, the subjects were in a state of no bleeding, (i.e. no clinical manifestation of an active bleed) and were eligible to continue in the trial according to the inclusion/exclusion criteria. Thromboelastographic measurements (*in vivo*) and other clinical laboratory assessments were recorded at baseline and for each post-dosing time point (15, 60, 120 and 240 minutes). A pre-dosing (baseline) blood sample was spiked with the equivalent randomisation dose of rFVIIa and analysed by thromboelastography (*ex vivo*) for each subject at the same *in vivo* post-dosing time points. Thromboelastographic measurements were taken using ROTEM® and TEG® instruments.

Number of Subjects Planned and Analysed

30 subjects were planned and randomised to treatment. Ten subjects were randomised to receive each treatment arm. All 30 subjects were included in the Intent-to-treat (ITT) and Per-Protocol (PP) populations.

Diagnosis and Main Criteria for Inclusion

The trial population consisted of male congenital haemophilia A and B subjects with or without inhibitors (FVII:C coagulation factor VIII clotting activity) or FIX:C (coagulation factor IX clotting activity) in a non-bleeding state. Subjects were at least 16 years of age and had given written informed consent prior to trial entry.

Test Product, Dose and Mode of Administration, Batch Number

rFVIIa/NovoSeven® (i.e. rFVIIa) 45 µg/kg, 90 µg/kg or 180 µg/kg i.v. infusion.
NovoSeven® Batch No.: rFVIIa 4.5 mg – Batch RR40060. Sterile Water (8.5 ml for infusion) – Batch PR40077.

Duration of Treatment

Each subject participated in the trial for a maximum of 18 weeks.

Reference Therapy, Dose and Mode of Administration, Batch Number

Not applicable to this trial.

Criteria for Evaluation – Efficacy

TEG® parameters measured *in vivo/ex vivo* prior to and at post-dosing time points:
- R time (reaction time), the latency time from start of analysis until initial clot formation
- K time is a measure of the speed to reach a certain level of clot strength
- α (α angle), is a slope drawn from the R time to the K time value and measures the rapidity of fibrin build-up and cross-linking, i.e. clot strengthening
- MA (maximum amplitude), is a direct function of the maximum dynamic properties (greatest vertical amplitude) of fibrin and platelet bonding
- LY30, measures the rate of amplitude reduction (lysis) 30 minutes after MA.

ROTEM® parameters measured *in vivo/ex vivo* prior to and at post-dosing time points:
- CT (clotting time), the latency time from start of analysis until initial clot formation
- CFT (clot formation time), is the measure of the time to reach a certain level of clot strength
- α (α angle), is a slope drawn from the CT to the CFT value and measures the rapidity of fibrin build-up and cross-linking, i.e. clot strengthening
- MCF (maximum clot firmness), is the greatest vertical amplitude
- LI60 (lysis index), measures the rate of amplitude reduction 60 minutes after CT.

Other laboratory measurements taken prior to and at post-dosing time points:
- Activated coagulation factor VII (FVIIa activity), coagulation factor X (FX:C), fibrinogen, number of platelets and mean platelet volume, prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Criteria for Evaluation – Safety

Serious adverse events (SAE) and treatment related adverse events (AE)

Statistical Methods

The ITT analysis set comprised all subjects who received trial product. All subjects in the ITT analysis set who did not violate the protocol in a manner judged to affect the efficacy evaluation were included in the PP analysis set. Intra-subject variability was assessed for TEG® and ROTEM® parameters. Intra-subject variation was presented with 95% confidence limits. The relationships between different doses of rFVIIa and the TEG® and ROTEM® parameters measured as a change from baseline were evaluated using linear regression models. Correlations between the thromboelastographic parameters from TEG® and ROTEM® for each time point were illustrated graphically. Estimations of inter-subject variability of TEG® and ROTEM® parameters at baseline and at all time points (15, 60, 120 and 240 minutes) were assessed; mixed models were used. Correlations between changes compared to baseline of *in vivo* and *ex vivo* thromboelastograph parameters were assessed by scatter plots. The correlation between thromboelastographic parameters and FVIIa activity, FX:C, fibrinogen, number of platelets and mean platelet volume, PT and aPPT were evaluated by Pearson correlation and Spearman rank-order correlation and scatter plots. Adverse events were listed.

Demography of Trial Population

The median age was 32 years (range 18-64 years). Median weight and height was 74.00 kg and 175.00 m, respectively. The majority of subjects were Caucasian (73.3%), and 26.7% were of unknown ethnic background. Baseline demographic characteristics were similar between the three treatment groups.

Efficacy Results

Large intra/inter-subject variation (ISV) was demonstrated for all TEG® and ROTEM® parameters.

Summary of TEG® Intra-Subject Variation

TEG® Parameter	**ISV**	**95% CI**	**CV (%)**	**ISV**	**95% CI**	**CV (%)**
	in vivo			*ex vivo*		

R time	255430	[213222; 311614]	29-69	70990	[56865; 91150]	28-35
K time	22535	[18493; 28072]	44	10394	[8236; 13531]	29
α-angle	119.77	[99.70; 146.63]	33-51	70.68	[56.60; 90.77]	22
MA	109.61	[91.28; 134.11]	27-34	39.32	[31.47; 50.55]	16
LY30	255.68	[212.39; 313.78]	53-75	69.51	[55.60; 89.41]	52

Summary of ROTEM® Intra-Subject Variation

ROTEM® Parameter	**ISV**	**95% CI**	**CV (%)**	**ISV**	**95% CI**	**CV (%)**
	in vivo			*ex vivo*		
CT	158858	[133654; 191971]	27-58	57401	[46095; 73467]	31
CFT	38028	[31476; 46873]	39-60	12149	[9656; 15755]	30-35
α-angle	148.32	[123.81; 180.95]	24-31	73.75	[58.92; 95.01]	18
MCF	88.57	[74.37; 107.30]	21-27	29.68	[23.76; 38.12]	12-13
LI60	415.22	[348.28; 503.61]	46-97	179.08	[143.67; 229.49]	28

- Intra-subject variation was larger for inhibitor subjects than for non-inhibitor subjects mainly for the TEG® method.
- Less intra-subject variation *ex vivo* compared to *in vivo* was observed for the TEG® and ROTEM® parameters.
- Treatment effect was shown for all ROTEM® and TEG® parameters, except K time for all subjects. There was a more pronounced treatment effect for inhibitor subjects compared to non-inhibitor subjects.
- Positive correlation demonstrated between the TEG® and ROTEM® parameters, but no agreement shown.
- Small correlations were observed between some TEG® and ROTEM® parameters with FVIIa activity, and PT.
- Moderate correlations were observed between all TEG® and ROTEM® parameters with aPTT.

Safety Results

- Only one subject in the 90 µg/kg treatment group (10% of subjects in 90 µg/kg treatment group) experienced three non-serious treatment related adverse events during the first treatment infusion.
- No deaths, serious adverse events, severe adverse events or withdrawals were reported.
- Overall, treatment with rFVIIa was well tolerated; there were no safety concerns with rFVIIa.

Conclusions

- Large intra/inter-subject variability was demonstrated in all thromboelastograph parameters limiting usability of these instruments in clinical trials.
- Intra-subject variation was larger for inhibitor subjects than for non-inhibitor subjects mainly for the TEG® method.
- Less intra-subject variation was demonstrated *ex vivo* compared to *in vivo*.
- A significant treatment effect (p-value <0.05) was observed for all ROTEM® and TEG® parameters, except K time for all subjects.
- Positive correlation demonstrated between the TEG® and ROTEM® parameters, but no agreement shown.
- Small correlations were observed between some TEG® and ROTEM® parameters with FVIIa activity and PT.
- The safety profile of rFVIIa indicated no safety concerns.

The trial was conducted in accordance with the Declaration of Helsinki as amended by the 52nd General Assembly in October 2000[1] and International Conference of Harmonisation Good Clinical Practice (May 1996)[2].