SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 1 February 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Nonafact is a plasma-derived factor IX product. The approved indication is "Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency)".

Factor IX is a single chain glycoprotein with a molecular mass of about 68,000 Dalton. It is a vitamin K-dependent coagulation factor and is synthesised in the liver. Factor IX activity is greatly reduced in patients with haemophilia B and therefore substitution therapy is necessary. Haemophilia B is a sex-linked recessive bleeding disorder that is 4 to 8 times less common than haemophilia A. The incidence is of about 1-2 per 100,000 in the population, occurring almost exclusively in males.

In the last three decades several types of plasma-derived coagulation products containing factor IX have been developed. The first generation of products, prothrombin complex concentrates, contain coagulation factors II, VII and X in addition to factor IX. The second generation of factor IX products have a reduced content of factor II, factor VII and X. Further product improvement has been achieved by the introduction of immuno-affinity chromatography, which allows for the production of very pure factor IX. Recently recombinant factor IX has been licensed. The availability of alternative products allows the selection of the optimal product for the individual patient. Potential risks of replacement therapy for bleeding episodes in patients with haemophilia B are transmission of blood-borne viral infections, thrombosis and development of inhibitors.

Nonafact is an immuno-affinity purified factor IX product. The manufacturing process includes solvent/detergent treatment for the inactivation of enveloped viruses such as HIV, hepatitis B and C viruses, and nanofiltration for the removal of viruses including non-enveloped viruses such as hepatitis A virus and parvovirus B19.

In February 1996 a "Note for Guidance to assess efficacy and safety of human plasma-derived factor VIII:C and factor IX:C products in clinical trials in haemophiliacs before and after authorisation" (CPMP/198/95 Final) came into operation. Clinical trials for the evaluation of Nonafact have been designed according to this CPMP Note for Guidance. A revised Note for Guidance CPMP/BPWG/198/95, rev 1 on "The Clinical Investigation of Plasma-Derived Factor VIII and IX Products" came into operation in April 2001. To support the application, the applicant submitted the results of three clinical studies with Nonafact performed in previously treated patients.

Abbreviations used in this report

ALT : alanine aminotransferase
ASAT : aspartate aminotransferase
HAV : Hepatitis A virus
HBsAg : Hepatitis B surface antigen
HBV : Hepatitis B virus
HCV : Hepatitis C virus
HIV : human immunodeficiency virus
HTLV : human T cell lymphotropic virus
PTP : previously treated patient
PUP : previously untreated patient
SD : solvent /detergent

2. Chemical, pharmaceutical and biological aspects

Composition

Nonafact is a freeze-dried powder in presentations of 500 and 1000 IU factor IX per vial, to be reconstituted with 5 or 10 ml of water for injections respectively.

Ingredients		Conc.	Function	Quality
Active ingredient	factor IX (IU/ml)	100		
Auxiliary substances	Histidine (mmol/l)	15	Stabilizer	Ph.Eur.
	Sucrose (mmol/l)	151	Stabilizer	Ph.Eur.
	Sodium chloride (mmol/l)	75	Isotonicity	Ph.Eur.
	Water for injections	q.s.		Ph.Eur.

The product is provided in colourless glass vials (Type I glass Ph. Eur.) of 8 ml (500 IU) or 20 ml (1000 IU). Water for injections is provided in colourless glass vials (Type I glass Ph. Eur) of respectively 11 or 20 ml. All vials of the product are closed with a bromobutyl rubber (Ph.Eur.) freeze-drying stoppers, sealed with an aluminium 'combi' cap. Vials water for injections are closed with rubber (Ph.Eur.) stoppers and sealed with an aluminium 'combi' cap.

Active substance

Source material

The information concerning the source material is submitted in a Plasma Master File (PMF). This PMF is in compliance with EC Guideline III/5272/94.

Plasma used for the manufacturing of Nonafact is derived from voluntary, non-remunerated donors meeting the criteria of non-remunerated donors as accepted by the Council of Europe. Criteria for the selection and acceptance of donors are in accordance with the relevant European and International recommendations. The plasma is supplied by the blood banks of Stichting Sanquin Bloedvoorziening (abbreviated: Sanquin) in the Netherlands. Blood banks are audited by the Dutch Health Inspectorate and by CLB, Products Division (a division of Sanquin).

All individual donations are tested for viral markers (HBsAg, anti-HIV 1/2, and anti-HCV) using commercially available kits. In addition, donations are tested for anti-HTLV I/II and antibodies against Treponema pallidum. Only donations tested negative for all these markers are used for the manufacturing of Nonafact. In the Netherlands, an ALT test is not performed on the donations. According to the CPMP position paper on ALT testing (CPMP/BWP/385/99), there is no scientific basis for objecting to the use of plasma for fractionation collected without ALT testing.

Plasma pools are tested for anti-HCV, anti-HIV 1/2 and HBsAg. Pools are also tested for HCV RNA.

All donations are traceable from the moment of collection until the final marketing and use of blood products prepared from them and vice-versa. Measures with respect to the post-collection information system are in compliance with CPMP/BWP/269/95 rev. 3 "Note for guidance on plasma-derived medicinal products".

A list of blood bags (all CE marked) used by the different blood banks is submitted. The type of blood bag, manufacturer, and anticoagulant is indicated.

Plasma collection, isolation from whole blood donations, methods of freezing, storage and shipping conditions and specifications are in compliance with the Ph. Eur. monograph "Human plasma for fractionation". Specifications for plasma are submitted.

Purification

The whole process is performed at CLB, Products Division in Amsterdam, the Netherlands. The starting material for the production of Nonafact is fresh-frozen plasma, which is firstly subjected to cryoprecipitation using conventional techniques. Factor IX is captured from the cryo-depleted plasma by adsorption to an anion exchange chromatographic matrix (first chromatographic step) from which, after washing, a factor IX-containing "3F-concentrate" is eluted. This factor IX fraction is diafiltered and concentrated and, after filtration, stored at ≤ - 25 °C.

3F-concentrate is thawed and S/D treated during at least 13 hours using TNBP and Tween-80. Factor IX is purified from the S/D-treated 3F-concentrate by immuno-affinity chromatography using monoclonal antibody CLB-FIX D4 coupled to Sepharose (second chromatographic step). After extensive washing to remove contaminants, the factor IX containing fraction (D4 eluate) is eluted with a chaotropic elution buffer. The eluate is filtered by dual nanofiltration using a pore size of 15 nm.
The nanofiltered factor IX solution is subjected to a third chromatographic step to remove not only mouse protein due to possible leakage from the monoclonal antibody matrix and the chaotropic components, but also possibly degraded factor IX. This is achieved by thorough washing of the matrix. Following elution of the bound factor IX the eluate ("third eluate") is concentrated and stored at ≤ - 25 °C.
Cleaning, sanitization, regeneration procedures and storage of relevant columns are described and re-use has been validated.

Active substance characterisation
Effective separation between factor IX and the other blood clotting factors II, VII and X is accomplished by the immuno-affinity chromatography process. This step also distinguishes between intact factor IX and activated factor IX (factor IXa). The specific activity of at least 200 IU/mg protein for this factor IX preparation is four times the requirement stated in the Ph. Eur. monograph, and the content of other coagulation factors is far below the Ph. Eur. limits.
Traces of high molecular weight proteins are present. No interference of these trace amounts with the biological activity of factor IX is expected. No factor IX aggregates are present.

Specification of active substance
An appropriate specification is set for the final concentrate.

Batch to batch consistency
The full scale production process was validated through compilation of process results of batches manufactured during the development phase in 1995. Additional validation data were obtained after introduction of changes in the manufacturing process by the production of new consistency batches of both 500 and 1000 IU fillings in 1999.
Comparison of the in-process results of the consistency batches of 1999 with those of the consistency batches manufactured in 1995 shows that the introduction of process modifications has no significant influence on the composition of intermediates and final product.

Stability of the active ingredient
Stability studies were performed with 3F-concentrate and final concentrate to justify the storage of these two intermediates.

Other ingredients

Excipients are of Ph. Eur. quality.

Product development and finished product

Product development
It was demonstrated that the formulation chosen gives the best overall product characteristics in terms of moisture content, appearance, reconstitution, osmolality, protein stability and activity.
Qualification batches produced in 1995 were used for shelf-life and pre-clinical studies. There are no changes in the formulation between the qualification batches and the formulation of Nonafact for marketing.

Clinical trial formula
Full QC results of all batches used for clinical studies are provided. All batches complied with the specifications. No significant differences were seen between batches produced for the pre-clinical studies, stability studies, clinical studies and the consistency batches.

Manufacturing process
Formulation and filling is performed at CLB, Products Division in Amsterdam, the Netherlands. The formulated bulk is manufactured from the final concentrate.
The solution is sterilised by filtration through a 0.22 µm membrane filter.
After filling, the vials are fitted with freeze-drying stoppers, freeze-dried, capped, and stored at 2 - 8 °C.
Satisfactory information is provided on in-process controls and the overall manufacturing process.

Satisfactory information on the manufacture of water for injections by SVM[1] is provided.

Finished product specification
The specifications for the finished product are based on the Ph.Eur. Monograph 1223. Potency of the finished product is determined by a chromogenic factor IX assay. This assay is validated and compared with the pharmacopoeial method (one-stage clotting assay). The in-house standards (concentrate and plasma standard) are calibrated against the International Standards for Factor IX concentrate and plasma (WHO standards).
A specific factor IXa assay has been developed. The principle of the factor IXa assay is the same as for the chromogenic assay for factor IX with the first activation step omitted.
An ELISA-based method was developed as an identity test since the precipitation method recommended by the Ph.Eur. is not suitable for this product. Tests for process related impurities (TNBP, Tween 80 and chaotropic components) are not included in the finished product testing. This is sufficiently justified by the retrospective analyses of 32 batches and by the routine monitoring of those impurities in the final concentrate. Mouse IgG is determined routinely on the finished product. The suitability of the methods used was sufficiently demonstrated.

Batch analyses
Qualification batches used in shelf-life and preclinical studies, batches used in clinical studies and consistency batches all complied with the finished product specification.

Finished product stability
To justify the claimed shelf life of 24 months at 2°C-8°C results of real time stability studies of 500 IU batches and 1000 IU batches (produced in 1995 and 1996 respectively) were submitted.
In addition, a new study with 500 IU batches and 1000 IU batches, manufactured on routine production scale, was started in 1999. For the 1999 batches, the results for 12 months storage at 2°C-8°C were presented. Based on the results submitted, a shelf-life of 24 months at 2°C-8°C is accepted. The Company is carrying out ongoing stability studies.

A shelf-life of 5 years is accepted for the water for injections based on the stability data submitted by the company. Ongoing stability studies are performed.

Stability reconstituted product
According to the SPC the product should be administered immediately for microbiological reasons. Chemical and physical in-use stability has been demonstrated for 3 hours at a temperature of 21°C.

Immuno-affinity matrix
A selective immune-affinity chromatography step is included in the purification process of Nonafact. Development, characterisation, fermentation and purification of this monoclonal antibody are described in sufficient detail.
The mouse monoclonal antibody CLB-FIX D4 is specific for non-activated FIX; it distinguishes between intact factor IX and factor IX that has been cleaved at position Arg 145, thus allowing the selective elution of non-activated factor IX.
Fermentation is carried out in serum-free medium. The purification process includes three different chromatographic steps: affinity chromatography, cation exchange chromatography and gel filtration. The eluate resulting from the affinity chromatography step is subjected to a solvent/detergent viral

[1] Stichting tot Bevordering van de Volksgezondheid en Milieuhygiene (Foundation for the Advancement of Public Health), Bilthoven, the Netherlands

inactivation step. Product containing fractions from the gel filtration step are pooled and subjected to nanofiltration. Consistency of production is demonstrated and appropriate specifications are set. Production of the immuno-affinity matrix is adequately described.
The stability of the monoclonal antibody and of the immuno-affinity matrix has been demonstrated in sufficient detail.

Viral safety and safety with respect to TSEs

A list of materials falling within the TSE (transmissible spongiform encephalopathy) guideline has been submitted. Applications for Certificates of Suitability have been made for these materials.

Four stages of the production process were separately assessed for their ability to remove and/or inactivate viruses: SD treatment, immuno-affinity chromatography, dual nanofiltration and the final chromatography step. The virus validation studies were performed in compliance with the CPMP for Guidance on virus validation studies. The choice of the viruses was appropriate. Robust inactivation of enveloped viruses (bovine viral diarrhoea virus (BVDV), HIV (human immunodeficiency virus) and pseudorabies (PSR) virus) was demonstrated for the S/D step.
Validation of the dual nanofiltration step was investigated using a range of enveloped and non-enveloped viruses including HAV (hepatitis A virus), canine parvovirus (CPV) and encephalomyocarditis virus (EMC). Immunoaffinity purification was shown to contribute to virus removal. The final chromatography step makes only a minor contribution to the overall viral safety of the product. The virus inactivation/removal capacity of the production process of Nonafact is sufficiently demonstrated.
The virucidal effect of the column sanitisation procedures is sufficiently demonstrated.

A summary of the viral reduction factors found in the manufacturing process of Nonafact is given below:

Process steps studied		Reduction factor (log) HIV	BVDV	PSR	EMC	CPV	HAV
A Incubation with SD chemicals (TNBP/ Tween 80)		>7.3	>5.8	>7.1	ND	ND	ND
B Immunoaffinity	4 ºC	>3.9	≥2.1	>5.1	≥3.6	≥3.9	≥1.6
Chromatography	12 ºC	≥ 5.2	≥1.7	>5.7	ND	>4.5	>3.1
C 15 nm nanofiltration	4 ºC	>4.7	>5.1	>5.3	>6.2	>4.9	>6.2
	12 ºC	>5.1	>5.0	>6.3	ND	>4.7	>5.9
D Final	4 ºC	ND	ND	ND	ND	1.9	1.5
Chromatography	12 ºC	ND	ND	ND	ND	1.6	1.5
Total reduction factor	4 ºC	>15.9	>13.0	>17.5	>9.8	>10.7	>9.3
	12 ºC	>17.6	>12.5	>19,1	ND	>10.8	>10.5

ND: not done

Monoclonal antibody CLB-FIX D4
The cell bank system has been tested for the presence of adventitious virus, retrovirus, Mycoplasma, bacteria, yeast and fungi. No evidence for the presence of adventitious agents was detected. At the end of every fermentation run, cells are tested for viruses. The following steps of the purification process have been validated for the virus removal/inactivating capacity: affinity chromatography, S/D treatment and nanofiltration. The reduction factors obtained are considered sufficient.
The virucidal effect of the column sanitisation procedures is sufficiently demonstrated.

Discussion on chemical, pharmaceutical and biological aspects

The quality of the product is considered satisfactory on the basis of the submitted data and the agreed follow-up measures. In particular, viral safety measures include appropriate donor selection, testing of donations and plasma pools for viral markers, and viral inactivation/removal procedures in the manufacturing process.

3. Toxico-pharmacological aspects

Pharmacodynamics
No studies of pharmacodynamic effects in animals were conducted. Animal data are not needed because human plasma-derived factor IX has a well-established physiologic effect in humans.

The thrombogenic potential of Nonafact was studied in the Wessler stasis model in rabbits and a non-stasis model in guinea pigs. In both models, Nonafact displayed considerably less thrombogenic potential as compared with conventional prothrombin complex concentrate. In the Wessler stasis model, Nonafact and a reference human plasma-derived factor IX product showed similar thrombogenic potential.

The thrombogenicity assays were conducted in compliance with GLP regulations.

No further safety pharmacology studies were conducted. Animal data are not needed because human plasma-derived factor IX has a well-established physiological effect in humans.

Pharmacokinetics
No animal studies have been conducted. Such studies are not necessary because of the human origin of factor IX, the relatively long history of its clinical use, and the evaluation of the pharmacokinetic aspects of Nonafact in clinical trials. Moreover, Nonafact displays a similar elimination half-life as compared to other factor IX products.

Toxicology

Acute toxicity, repeated dose toxicity, reproductive toxicity, mutagenicity, carcinogenicity
As factor IX is a normal constituent of the human body, its clinical use (which is to obtain a physiological concentration of the constituent) is not expected to be associated with toxicity. Products containing factor IX have been used for decades and the clinical experience of their use is therefore extensive. Furthermore, due to the protein character of factor IX, a full investigation of the toxicological parameters in animals is not feasible. Therefore, no animal studies have been performed to assess toxicity of factor IX.
The toxicity related to impurities has been addressed sufficiently.

Local tolerance
No animal studies have been performed. Clinical experience provides no hint for local effects caused by Nonafact.

Discussion on non-clinical aspects

Plasma coagulation factor IX is a normal constituent of human plasma. Factor IX in the product, therefore, behaves like endogenous factor IX. Conventional animal toxicity studies and mutagenicity studies with plasma coagulation factor IX would not provide meaningful information and therefore were not carried out. In pharmacodynamic studies in rabbits and guinea pigs, the thrombogenicity of Nonafact was shown to be minimal.

4. Clinical aspects

The approved indication is "Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency)".

The submitted clinical trials to investigate efficacy and safety of Nonafact were carried out in 26 previously treated patients (PTPs) with haemophilia B for treatment and prevention of bleeding (study KB96003) and in 8 patients undergoing elective surgical procedures (study KB96002). A subset of study KB96003 (study KB96001) investigated pharmacokinetics in 13 patients.
This number of patients exceeds the number of patients required according to the CPMP document "Note for Guidance to assess efficacy and safety of human plasma derived factor VIII:C and factor IX:C products in clinical trials in haemophiliacs before and after authorisation" (CPMP/198/95) which came into operation in February 1996 and is also in agreement with the revised document "Note for Guidance on the Clinical investigation of human plasma derived factor VIII and IX products" (CPMP/BPWG/198/95 rev. 1) which came into operation in April 2001.
The clinical trials were performed according to GCP.

Pharmacokinetics
Study KB96001 included 13 patients, age ≥18 years, with severe haemophilia B (factor IX ≤ 2%). Half-life and recovery were assessed in 13 patients in the beginning of the study with a second assessment in 5 patients after 6 months. Patients received a single dose of 25-75 IU/kg. Samples for factor IX activity were taken before injection of Nonafact and 15, 30 minutes, 1, 3, 6, 12, 30 and 48 hours after the transfusion. The results are reported in Table 1.

The half-life of factor IX reported in the literature ranges between 18 and 34 hours.[2,3] This variability is partly due to a difference in mathematical approach for assessing half-life. Model-independent methods for analysing single-dose pharmacokinetics[4] tend to result in lower half-life values than model-dependent (two-phase) models[5]. As illustrated in table 1, both mean terminal half-life and recovery remained stable over time. The only statistically significant difference was the model dependent mean terminal half-life, which showed an increase from 29.01 hours to 38.00 hours. The value of 38 hours is somewhat longer than expected based on the data from the literature. However, given the limited number of patients (5) and the large variability of the second half-life assessment, the apparent half-life prolongation is probably of no clinical relevance. Furthermore, the model independent analysis did not reveal any significant difference between the first and second assessment. These values are similar to the reported range for other plasma-derived or recombinant factor IX products (16-18 hours) using the same model.[6]

[2] *Thompson AR: Factor IX concentrates for clinical use. Semin-Thromb-Hemost. 1993; 19(1): 25-36*
[3] *Bjorkman S and Carlsson M. The pharmacokinetics of factor VIII and factor IX methodology, pitfalls and applications. Haemophilia 1997; 3: 1*
[4] *Longo G et al. Single-dose pharmacokinetics of factor IX evaluated by model-dependent methods. Eur J Haematol. 1987; 39: 426*
[5] *Lee ML et al A two-phase linear regression model for biologic half-life data. J Lab Clin Med 1990; 115: 745*
[6] *Poon et al. Comparison of the recovery and half-life of a high-purity factor IX concentrate with those of a factor IX complex concentrate. Transfusion 1995; 35: 319. White GC et al. Recombinant factor IX. Thromb Haemost. 1997; 78: 261*

Table 1: Half-life and recovery assessed in 13 patients in the beginning of the study and a second assessment in 5 patients after 6 months of follow-up (study KB96001).

	Start of study (N=13)	after 6 months (N=5)
mean terminal half-life: hours (SD)	model independent: 18.68 (1.99) model dependent: 29.01 (9.66)	model independent: 17.94 (1.85) model dependent: 38.00 (16.22)
Recovery: U/ml per IU/kg factor IX infused (SD)	0.011 (0.002)	0.011 (0.003)

Clinical efficacy

Three clinical studies, all carried out in previously treated haemophilia B patients have been submitted. The studies were carried out in patients with various degrees of haemophilia B, mostly severe, and were enrolled from 5 participating centres in the Netherlands (n=4) and Poland (n=1).

In *study KB96003* a total of 26 previously treated patients with severe haemophilia B (factor IX ≤ 2%) were included (14 patients in the Netherlands and 12 in Poland). None had an inhibitor to factor IX at the time of inclusion. The primary objective was to determine the in vivo recovery and half-life in a subset of patients (n=13) (KB96001), the secondary objective was the demonstration of viral safety and clinical efficacy of Nonafact. Thrombogenicity of Nonafact was also investigated. Patients were also followed for development of factor IX inhibitors.

Response per infusion for the treatment of major or life-threatening bleeding episodes was assessed subjectively using a four-point scale ("excellent", "good", "moderate" and "none"). HIV seropositive patients and CD4 lymphocytes count ≤400/µl were excluded. The mean age was 32.9 years. Four patients (all from the Netherlands) used Nonafact as prophylactic treatment, the 22 other patients on demand (in case of a bleeding or in case of planned, extensive, physical exercise, including intensive physiotherapy at the Polish haemophilia treatment centre). The administration of Nonafact was supervised by the investigator or a designate. Part of the study consisted of a follow-up period of at least 6 months and with a minimum number of 10 exposure days to Nonafact in each patient. The mean number of exposure days to Nonafact at visit 5 (18-month follow-up) was 71.8 days (range 16-174 exposure days). The mean total dose of Nonafact transfused between visit 1 and 5 was 108,129 IU.

During the 18-month follow-up period, 12 major or life-threatening bleedings occurred in 9 patients (5 muscular bleedings, 2 gingival bleedings, 4 surgeries and 1 trauma capitis). The four surgeries consisted of 2 minor surgeries (dental extraction and surgical removal of verrucae seborrhoica) and 2 major surgeries (abdominal surgery and a total hip replacement and replacement of both knees). In all cases but one, which was considered as good, the clinical effect of Nonafact was judged by the investigator as excellent.

A total of 780 minor bleedings were reported during the study period; 640 (82%) of these minor bleedings were stopped after a single transfusion of Nonafact, 108 (13.8%) haemorrhages required 2 infusions, 21 (2.7%) required 3 infusions, and 12 (1.5%) 4 or more infusions. Only 4 patients used Nonafact prophylactically. Between visit 1 and visit 5 (18 months), Nonafact transfusions were required in all patients in the follow-up study because of minor bleedings, except for 2 patients, who both used prophylactic treatment already before the study.

At the time of the response to the list of questions, results for the 12 patients treated in Poland were available for the completed 36-month period of study. One patient was lost to follow-up after the 18-month visit, so no data were available for visit 6 (24 months) and 7 (36 months). The mean number of exposure days to Nonafact between visit 1 and 7 was 126.8 days (range 16-175 exposure days). The mean total dose of Nonafact transfused in the same time period was 131,899 IU.

During this 36-month period, nine major bleedings occurred in seven patients. These comprised muscular bleedings in five cases, gingival bleeding, haematuria, bleed undergoing surgery (total hip replacement and an arthroplasty of both knees) and dental extraction each in one case. None of the bleedings was considered related to the study drug and in all cases but one the clinical effect of Nonafact transfusions was judged excellent by the investigator. In the one case it was considered good.
A total of 1046 minor bleedings was reported; 77% of these minor bleedings were stopped after one single transfusion of Nonafact.

The 14 patients treated in the Netherlands will be followed until the marketing authorisation for Nonafact is granted and an interim report after 36 months will be submitted. The mean dosages used in the patients treated in Poland were lower than those used in the Dutch patients, although still efficacious.

Study KB96002 was an open, multicentre study of 8 haemophilia B patients undergoing a total of 11 surgical interventions. One patient had mild haemophilia (factor IX of 2%) and 7 had severe haemophilia. The mean age was 45.6 years. The objective was to show the clinical efficacy of Nonafact in patients with haemophilia B undergoing a surgical procedure. The type of surgery is indicated in table 2. Patients were treated with a single i.v. bolus of 40-60 IU/kg before a dental extraction and a single i.v. bolus of 60-90 IU/kg for other surgical interventions. Further dosing depended on the factor IX level assessed in the plasma. During substitution therapy the plasma level should be maintained at least at 50%. The criteria for evaluation was achievement of a satisfactory haemostasis (to be assessed by the investigator), the amount of blood loss and the transfusions of blood components required. Furthermore, samples for the determination of thrombogenicity were drawn before the first transfusion of Nonafact and 5 and 30 minutes thereafter. In addition, adverse events and laboratory variables were evaluated.

From a total of 11 surgeries, only two resulted in blood loss.
Seven of the surgical procedures were dental extractions in 4 patients, prior to which only one transfusion with Nonafact was given. No measurable amount of blood loss was detected in any of the patients undergoing dental extraction. However, in all dental extractions, tranexamic acid was concomitantly administered. Although this is a common approach in managing oral bleeding in haemophilia patients, evaluation of efficacy of Nonafact in these patients is more difficult to establish and cannot be solely attributed to the transfusion of Nonafact.
The patient who underwent a pancreas cyst drainage received 4 Nonafact transfusions in 4 days, the patients with a cholecystectomy received 2 transfusions per day resulting in a total of 18 Nonafact transfusions in 10 days. The patient with 'removal of varicose veins' received 2 transfusions during the first 8 days and 1 transfusion per day during the last 4 days. The 'arthroplasty' patient had 2 transfusions per day during the first 19 days and 1 transfusions per day during the next 10 days. Blood loss was observed for the 2 major surgeries (life-threatening surgeries). The amount of blood loss during 'removal of varicose veins of the left lower limb' was 0.15 litre and for the 'total arthroplasty of left and right knee and total hip replacement right' a blood loss of 1.2 litre was recorded. No post-surgical bleedings occurred. One patient was treated with fractionated heparin to prevent the risk of thrombo-embolic events after orthopaedic surgery. In all cases, the circulating level of factor IX, 30 minutes after transfusion was higher (mean 88.4% for the dental extractions and 53%, 61%, 93% and 82% in the other surgical procedures) than the recommended minimum level. Also the level of circulating factor IX 30 minutes after Nonafact transfusion was higher then would be expected considering the dose-calculation formula. Values of haematological parameters outside normal ranges (such as activated partial thromboplastin time (APTT)) could be explained by underlying disease. Other abnormal coagulation variables were not clinically relevant.

Table 2: type of surgical procedures (studies KB96002)

Surgical procedure	number of surgical procedures (number of patients)
Minor surgery Dental extraction	7 (4)
Other surgery (major): pancreas cyst drainage cholecystectomy	2 (2)
Other surgery (life-threatening): removal of varicose veins in left lower limb combined orthopaedic procedure	2 (2)

Clinical safety

Adverse events
In the first 18-month period of study KB96003, including 26 patients with severe haemophilia B, 28 adverse events occurred, of which 12 were major or life threatening bleedings. According to the investigator 3 of the 28 adverse events were possibly related to the study drug. These adverse events were 'flushes after drug administration' during the pharmacokinetic sub-study in one patient and 'elevation of bilirubin' at visit 3 in one patient, and 'elevation of bilirubin and ALT and ASAT' at visit 3 in another patient. The last two adverse events could be explained by the HCV positive status of the patients. See also 'Viral safety'.
In the surgery study KB96002 two adverse events were reported (pyrexia and decrease of Hb after arthroplasty in one patient); both events were not considered related to the study drug.

Vital signs
During the pharmacokinetic study (KB96001), vital signs (blood pressure, pulse and temperature) were measured 7 times per pharmacokinetic assessment. There were no clinically relevant abnormalities recorded.

Viral safety:
In study KB96003, 26 patients with severe haemophilia B were treated with Nonafact. At the start of the study, 26 patients were HCV seropositive and 25 patients were seropositive for parvovirus B19. Twenty-one of the 26 patients were either vaccinated for hepatitis B or were previously infected. Therefore, transmission of HCV, parvovirus and hepatitis B could not be evaluated. No patient was either HIV-1/HIV-2 seropositive prior to study entry. Results at the 18-month study time-point are indicated in table 3. No viral transmission of HAV, HIV-1 or HIV-2 was documented during follow up. All patients remained seronegative.
In the surgical study KB96002 virus serology was only performed at admission.

Table 3: Viral safety (study KB96003 including 26 patients)

	Start of study Seronegative	during follow up seronegative
HAV	20 patients	20 patients
HBsAg and HBs antibodies	5 patients (21 patient were either vaccinated or had a hepatitis B infection before the start of study). Of these 5 patients, only one patient was negative for HBsAg and HBs antibodies at the start of study; 4 patients were HBsAg positive	one patient was evaluable: and remained HBsAg and HBs antibody negative.
HCV	0 patients	not evaluable
HIV-1/2	26 patients	26 patients
Parvovirus -B19	0 patients (25 of 26 were tested)	not evaluable

Thrombogenicity
In the pharmacokinetic study KB96001, including 13 PTPs with severe haemophilia B (factor IX ≤2%), the thrombogenicity of Nonafact was examined by the assessment of prothrombin fragment 1+2 before Nonafact administration, and at 30 minutes, 1 and 3 hours after transfusion. Fragment 1+2 levels were all within the 95% confidence interval of normal, except one measurement, which was slightly higher than the other measurements. No thromboembolic events occurred.
In study KB96003 including 26 patients (part of which are the 13 patients of the pharmacokinetic study KB96001) no thromboembolic events occurred.
In the surgical study KB96002 eight patients were included undergoing 11 surgical procedures. Fragment 1+2 values (measured 5 and 30 minutes post-administration) were all within the normal range, although one measurement was slightly higher than the other measurements. No thrombogenic event occurred in any of the patients during follow up.

Immunogenicity
In study KB96003 all 26 PTPs were screened for the development of an inhibitor at each visit. Antibodies to factor IX antibodies were not detected in any of the patients.

Safety results from the completed 36-month study of patients treated in Poland
The results of the completed study for the 12 patients treated in Poland in study KB96003 were available at the time of the response to the list of questions.

Adverse events
A total of 12 adverse events were recorded, 10 of which were considered unrelated to Nonafact. According to the investigator, two adverse events, 'hyperbilirubinemia' and elevation of bilirubin, ALT and ASAT, were possibly related to the study drug. These adverse events could be explained by the HCV positive status of the patients.
One adverse event, a bleeding of the M. Iliopsoas was considered serious.

Thrombogenicity
No thromboembolic events occurred in this study.

Immunogenicity
Anti-factor IX levels were not detected in any of the patients during the study.

Viral transmissions
Since 9 patients were positive for HBs antibodies and three patients were positive for HBsAg at the beginning of the study, Hepatitis B seroconversion could not be studied. All 12 patients were negative

for HAV antibodies and HIV-1 and HIV-2 antibodies at visit 1. No seroconversion occurred during the study with respect to HAV and HIV 1+2.

Discussion on clinical aspects

Half life (model independent: 18.68 hours) and *in vivo* recovery (0.011 U/ml per IU/kg factor IX infused) for the 13 patients investigated was shown to be in the reported range for other plasma-derived or recombinant factor IX products and remained stable after 6 months in 5 tested patients.

The clinical experience in the pharmacokinetic and the follow-up study referred to 1867 cumulative exposure days (average of 71.8 days per patient) over an 18-month study period. Submitted data on the clinical response in on demand treatment regimens (study KB96003), including a sufficient number (N=26) of previously treated patients with severe haemophilia B, give reassurance of the efficacy of Nonafact as 82% of a total of 780 minor bleedings stopped after a single transfusion of Nonafact. A total of 9 patients had a major or life-threatening bleeding. In all cases but one, the clinical effect of the Nonafact transfusions was judged by the investigator as excellent. In the one remaining case the effect was considered to be good. Furthermore the reported efficacy in major surgical procedures (including major orthopaedic surgery, pancreas cyst drainage, cholecystectomy, and removal of varicose veins) indicate that Nonafact is effective. Unfortunately, in all dental extractions (7 procedures in 4 patients), tranexamic acid was concomitantly administered. Therefore, in these cases haemostatic efficacy cannot be solely contributed to the transfusion of Nonafact.
Efficacy was further supported by the 36-month study period data on 11 patients in study KB96003 treated in Poland.

The number of patients tested for vital signs, adverse events, thrombogenicity and immunogenicity meets the requirements in the guideline CPMP/BPWP/198/95 Final (February 1996).

The submitted data of studies KB96003 and KB96002 give reassurance on the safety of the product with respect to thrombogenicity, although longer follow up is necessary to establish this conclusively. No thromboembolic events have been reported and adequate thrombogenicity tests (fragment 1+2) after Nonafact infusion in 13 patients included in the pharmacokinetic trial and in 8 patients undergoing surgical procedures did not show significant elevations.

The lack of high immunogenicity in PTPs is also established, as no antibodies to factor IX were reported in 26 PTPs, who received the product for a median of 71.8 days per patient (range 16-174 exposure days). In addition, no antibodies were detected in the 11 of these patients treated in Poland at the end of the 36-month study period (exposure days: mean 126.8, range 16-175 exposure days; mean total dose transfused: 131,899 IU.)

The company considers monitoring of development of circulating antibodies to murine immunoglobulins inappropriate, as it is estimated that 85% of severe haemophilia B patients in the Netherlands have already been treated with an immuno-affinity purified factor IX product. Furthermore the presence of such antibodies of the IgG type has been demonstrated in 7-10% of the normal donor population. These arguments are considered valid.

According to the Note for Guidance (CPMP/BPWG/198/95 rev.1), it is no longer considered appropriate to use clinical trials to investigate viral safety with regard to enveloped viruses. Moreover, the safety of the products with respect to non-enveloped viruses cannot currently be adequately evaluated in clinical studies. The viral safety data, provided by the applicant (in accordance with the former Note for Guidance (CPMP/198/95 final) and in line with normal clinical practice of monitoring patients), demonstrate that there was no transmission of HAV, HIV 1 or HIV 2. Transmission of HCV and Parvo B19 could not be investigated as all patients were antibody positive at the start of the study. Only one patient was HBV and HBsAg negative at study entry. As most haemophilia B patients will be vaccinated against hepatitis B, transmission of HBV virus cannot be evaluated in clinical studies.

The company intends to perform a prospective pharmacovigilance study post-authorisation including patients with mild, moderate and severe haemophilia B in accordance with CPMP/BPWG/198 rev. 1.

Since children may respond differently to adults, the company has committed to perform an open multicentre study including at least 12 children with haemophilia B under the age of six years regardless of prior treatment. A proposal for a study protocol has been submitted (KB200001). This study will be in accordance with the Note for Guidance CPMP/198/95, rev 1. In the meantime, the SPC includes a statement that there are insufficient data to recommend the use of the product in children less than 6 years of age.

No data on previously untreated patients (PUPs) have been submitted in accordance with the former version of Note for Guidance CPMP/198/95. In accordance with the CPMP guideline, the SPC includes a section stating the lack of experience in PUPs. After authorisation, the Company will include in the Periodic Safety Update Reports immunogenicity and viral safety data on any PUPs treated with Nonafact, of which the company becomes aware, or any PUP included in the study in children according to protocol KB200001.

5. Overall conclusion and benefit/risk assessment

- **Quality**

On the basis of the submitted data and the agreed follow-up measures, the quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Viral safety and batch to batch consistency have been documented and the relevant tests will be performed according to the agreed specifications.

- **Non-clinical (pre-clinical studies)**

Plasma coagulation factor IX is a normal constituent of human plasma. Conventional animal toxicity studies and mutagenicity studies with plasma coagulation factor IX would not provide meaningful information and therefore were not carried out. The thrombogenicity of Nonafact was shown to be minimal in pharmacodynamic studies in rabbits and guinea pigs. Toxicity related to impurities has been addressed sufficiently.

- **Clinical efficacy and safety**

Half life and *in vivo* recovery in the 13 patients investigated was shown to be in the reported range for other plasma-derived factor IX products and remained stable after 6 months in 5 tested patients. Submitted data on the clinical response in on demand regimens give reassurance of the efficacy of Nonafact. Furthermore, the reported efficacy in major surgical procedures indicates that Nonafact is effectiveThe submitted data give reassurance on the safety of the product with respect to thrombogenicity, although longer follow-up is necessary to establish this conclusively. No indication of immunogenicity, as measured by antibodies to factor IX, was detected in the 26 PTPs investigated. No transmission of HAV, HIV 1 or HIV 2 occurred.
A prospective pharmacovigilance study will be performed post-authorisation, in accordance with CPMP/BPWG/198/95 rev. 1, and seronegative patients will be monitored for parvovirus B19 and HCV status. Since children may respond differently to adults, an open multicentre study in children under the age of 6 years will be undertaken. There is no experience of the treatment of PUPs with Nonafact. This is indicated in the SPC. Immunogenicity and viral safety data on any PUPs treated with Nonafact that the company becomes aware of will be included in the Periodic Safety Update Reports.

Benefit/risk assessment

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Nonafact in the treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency) was favourable.