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DRAFT

GUIDELINE ON THE CLINICAL INVESTIGATION OF RECOMBINANT FACTOR VIII AND IX PRODUCTS

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GUIDELINE ON THE CLINICAL INVESTIGATION OF RECOMBINANT FACTOR VIII AND IX PRODUCTS

TABLE OF CONTENTS

EXECUTIVE SUMMARY

This guideline describes the information to be documented when an application for a marketing authorisation for recombinant Factor VIII (rFVIII) or Factor IX (rFIX) products is made for use in treatment and prevention of bleeding in patients with haemophilia A or haemophilia B. The guidance covers clinical trials and post marketing surveillance studies. Guidance is also provided for authorised products where a significant change in the manufacturing process has been made.

1. INTRODUCTION (background)

The purpose of this guidance is to provide applicants and regulators with harmonised guidance for applications for marketing authorisation for recombinant Factor VIII or Factor IX products.

2. SCOPE

The guidance covers clinical trials and post marketing surveillance studies. Quality aspects are outside the scope of this guideline.

3. LEGAL BASIS

This guideline has to be read in conjunction with the introduction and general principles (4) and part I of the Annex I to Directive 2001/83 as amended.

4. MAIN GUIDELINE TEXT

4.1 INTRODUCTION

In view of the high rate of transmission of blood-borne viruses by plasma-derived (pd) coagulation factor concentrates in the 1970s and early 1980s, there was considerable interest in the possibility of producing factors VIII and IX products by recombinant DNA technology. The structure of the factor VIII gene was elucidated in 1984, followed by the isolation of cDNA clones encoding the complete factor VIII sequence, and the *in vitro* expression of human factor VIII, in tissue culture. Since then commercial production of recombinant full-length and modified factor VIII as well as recombinant factor IX products have been accomplished for clinical use.

In plasma, factor VIII occurs as a heterodimer, consisting of a light chain (domains A3, C1 and C2), and a heavy chain (domains A1 and A2) and domain B seemingly lacking specific functions.

A comparison of pharmacokinetic parameters of rFIX and pdFIX indicated that the elimination half-lives were nearly identical whereas the *in vivo* recoveries were statistically different. Differences in sulfation and lack of phosphorylation in rFIX may account for the lower recovery of rFIX as compared to pdFIX.

The occurrence of an antibody against factor VIII, a so-called inhibitor, is the most important complication in haemophilia treatment. Inhibitors occur in up to about 30% of previously untreated patients (PUP) with severe haemophilia A, usually within the first 100 exposure days.

rFVIII products have also been associated with the development of inhibitors with a cumulative incidence of up to around 30%. These inhibitors have mainly been observed in previously untreated children, and approximately one third disappeared on continued treatment with the same product. It now appears that in cases in whom inhibitors occur, patient related factors (certain types of mutations in the factor VIII gene, the family history, ethnicity and possibly HLA-DR constitution) appear to be important determinants of inhibitor development. The immunogenicity of rFVIII has to be addressed, because in rFVIII products a heterogeneous protein pattern might occur due to differences in the posttranslational modifications of the proteins. Patients treated with rFVIII and rFIX products should be carefully monitored for the development of inhibitory antibodies by appropriate clinical observations and laboratory test.

Two inhibitor “outbreaks” occurred in the early 1990’s in previously tolerant patients who had been treated for a number of years following exposure to plasma-derived products subjected to a modified virus inactivation method. Hence, the incidence of inhibitor formation may be affected by the specific product used for treatment and its potential to result in alteration of factor VIII molecules,

'neoantigens'. It was apparent from this experience that the risk of inhibitor formation related to an individual product could be evaluated in previously treated patients (PTPs). PUPs should not be used for study of product related immunogenicity of products, since patients with a high degree of previous exposure appear to be a better suited study population.

Clinical trial data, addressing efficacy and safety with respect to immunogenicity and other adverse events, are required in an application for a marketing authorisation. In addition, the potential for thrombogenicity should be investigated in the case of factor IX products. This guideline describes the clinical trials required for authorisation with respect to rFVIII and rFIX products. These data are required for:

1. products for which an application for a marketing authorisation is to be submitted, referred to as 'new products' in the text and
2. authorised products where a significant change in the manufacturing process has been made (e.g. new purification procedures and/or omitting human or animal-derived proteins during manufacture).

The clinical trials described in this guideline should be performed according to the ICH Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95).

4.2 EFFICACY

In clinically evaluating human recombinant coagulation factors for the treatment of haemophilia A or B patients, the initial trial typically examines the pharmacokinetics of the principal active factor. Appropriate pharmacokinetic data (incremental recovery, half-life, area under the curve (AUC), and clearance) are the most important (surrogate) endpoints for efficacy of a new factor VIII/IX product. The International Society on Thrombosis and Haemostasis (ISTH) also provides guidance on pharmacokinetic studies. It could be useful to consult this guidance for advice when designing studies.

4.3 SAFETY

Safety aspects of factor VIII/IX products include viral safety, immunogenicity and other adverse events. For recombinant products the use of non-human cell-lines raises the possibility of different contaminants and altered immunogenic potential. For factor IX products thrombogenicity should also be considered a safety issue.

4.3.1 Adverse events

All adverse events occurring in relationship with any use of the product should be recorded and reported.

Product specific:

Development of hypersensitivity reactions to heterologous proteins (e.g. murine, bovine or hamster origin) with related adverse reactions.

4.3.2 Safety with respect to transmissible agents

4.3.2.1 Viral safety

The safety of recombinant products with regard to viral contamination can only be reasonably assured by the application of virus testing within the manufacturing process and assessment of virus inactivation and removal during the manufacturing process, according to the relevant guidelines (e.g. ICH 'Note for Guidance on quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin' CPMP/ICH/295/95).

4.3.2.2 Other transmissible agents

Similar principles to those outlined in 4.3.2.1 apply for safety with regard to other transmissible agents including TSE and other emerging pathogens. Manufacturers should follow the respective guidance documents and position statements. Information can be found in the section "Guidelines on

Recombinant Medicinal Products" on the EMEA website:
(http://www.emea.europa.eu/htms/human/humanguidelines/biologicals.htm).

4.3.3 Immunogenicity

In general, immunogenicity should be investigated prior to marketing authorisation and substantiated with post marketing surveillance studies.

4.3.3.1 Factor VIII products

The occurrence of antibodies against factor VIII is a major complication of haemophilia A treatment. The risk of inhibitor occurrence is higher in patients with severe haemophilia A than in patients with moderate and mild disease and also the genotype, ethnic background of the patient is relevant (high risk: inversions, large deletions or nonsense mutations of the factor VIII gene). In addition risk may be associated with commencing treatment in previously untreated patients or with changing of treatment or where the antigenicity of the product has been altered due to changes in the manufacturing process. Previously treated patients are the most suitable candidates to test the product-related immunogenicity of a factor VIII product.

Neutralising antibodies are the most important immunological topic and therefore the following aspects and basic principles should be followed:

- Inhibitor development should be studied in previously treated patients (>150 exposure days, suffering from severe haemophilia A with a FVIII level < 1%);
- The modified Nijmegen method of the Bethesda assay should be used. Validated testing should be performed in a centralised laboratory;
- A blinded inhibitor retesting using a second sample should be performed in a central laboratory;
- The definitions for thresholds are ≥0.6 BU for "a low titre" inhibitor and ≥5 BU for a 'high-titre' inhibitor;
- Preferably inhibitor testing should be performed when FVIII level has reached baseline;
- Conditions influencing FVIII inhibitor measurements should be screened and documented like chronic viral infections (e.g. HIV, HCV) or Lupus anticoagulant;
- Detailed patient characteristics should be available (e.g. ethnicity, family history, life style, general health status, infection status, type of FVIII gene mutation, reason for treatment, start of treatment, kind of treatment (on demand, prophylactic, continuous infusion));
- Recovery should be monitored.

4.3.3.2 Factor IX products

Haemophilia B is around 4 times less common than haemophilia A. The incidence of inhibitors in these patients following administration of factor IX is less common compared to the incidence found in haemophilia A patients. Inhibitors to factor IX have been demonstrated in approximately 4% of patients with severe haemophilia B. It has been observed that the occurrence of inhibitors is commonly associated with the total deletion of the factor IX gene. Unlike those with haemophilia A, patients with haemophilia B more often experience anaphylactic reactions to factor IX products in association with the development of inhibitors. Literature also reports on the occurrence of anaphylactic type reactions as well as the development of a nephrotic syndrome following immune tolerance therapy. These problems have been observed for plasma-derived as well as for recombinant factor IX products.

4.3.4 Thrombogenicity (Factor IX products)

Treatment with pdFIX products that contain factors II, VII and X has been associated with thrombosis. Factor IX products with higher purity and rFIX have displayed less risk of thrombogenicity. For new factor IX products, tests for markers of activation of coagulation should be carried out in post-infusion samples obtained in the non-bleeding state.

4.4 PRODUCTS FOR WHICH AN APPLICATION FOR A MARKETING AUTHORISATION IS TO BE SUBMITTED: 'NEW PRODUCTS'

4.4.1 Clinical trials with new recombinant factor VIII products

4.4.1.1 Efficacy

A pharmacokinetic trial should be performed in at least 12 subjects suffering from severe haemophilia A (factor VIII ≤1%). The study should record incremental recovery, *in vivo* half-life, area under the curve (AUC), and clearance in patients without inhibitors who are not actively bleeding. Patients should be at least 12 years of age and should not have received an infusion of any FVIII product for at least 4 days. Prior to the first administration of the new factor VIII product, half life of the previous product should be investigated in all patients. Samples for factor VIII activity determination should be taken before injection of 25-50 IU/kg of the factor VIII product and at 30 minutes, 1-3, 4-6, 7-9, 10-14, 20-26, 28-30 and 32-48 hours after the infusion. At least 3 different lots should be employed in the trial. Incremental recovery is determined as the peak level recorded 30 minutes after infusion and reported as [IU/ml]/[IU/kg]. According to the European Pharmacopoeia monograph for human coagulation factor VIII, potency assignments for factor FVIII products have to be performed with the chromogenic assay. Preferably the same assay should be used for analysis of the product and the patient's plasma.

It is very important to record the exact time post-infusion at which the actual samples were collected and to use these precise values in the analysis.

Patients taking part in the pharmacokinetic trial should continue treatment with the product for 6 months, and should be re-tested for the same pharmacokinetic parameters after 3-6 months using the same dose as in the first investigation.

Clinical efficacy of factor VIII should be evaluated in at least 50 PTPs (>12 years, > 150 exposure days (ED)), suffering from severe Haemophilia A (factor VIII ≤ 1%, CD4 > 200/μL). During an observation period of a minimum of 50 exposure days, clinical response should be assessed by the patients. Response should be assessed as "none", "moderate", "good" or "excellent" by the physician for those patients who were treated in hospital with the product for major bleeds. In addition, response will be determined by the physician in a minimum of 5 patients undergoing at least 10 surgical procedures, including efficacy of haemostasis, loss of blood, and requirements for transfusion.

For the assessment of clinical efficacy of factor VIII claimed in long-term prophylaxis, patients should be followed for 6 months for bleeding episodes, bleeding intervals and number of treatments.

Clinical efficacy should be assessed by calculating the consumption of factor VIII, expressed as number of infusions and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).

Continuous infusion

If a claim for continuous infusion therapy is requested, the study should be carried out in at least 12 severe haemophilia A patients (FVIII ≤1%) undergoing elective major surgical procedures.

Prior to surgery, a pharmacokinetic analysis in each individual should be performed to obtain, in particular, an estimate of clearance. The initial infusion rate could be based on the clearance as follows:

Clearance x desired steady state level = infusion rate (IU/kg/hr)

(if necessary plus a corresponding safety margin)

After the initial 24 hours of continuous infusion, the clearance should be calculated again every day using the steady state equation with the measured level and the known rate of infusion.

Efficacy and safety data during surgery and for at least 6 days thereafter should be submitted, including PK parameters with the description of the assay used, daily dosage of factor VIII with the

description of the administration method used, administration rate, consumption, haemostatic response and blood loss, transfusion requirements and local and systemic side-effects.

Pharmaceutical data on reconstitution and stability of the product should be provided in the Quality section of the dossier.

Immune tolerance

Any labelling claim for induction of immune tolerance in haemophilia A patients with inhibitors should be supported by clinical data conducted with the specific product.

4.4.1.2 Safety

Safety will be assessed in all patients receiving the factor VIII product during clinical trials including vital parameters. All adverse events in clinical studies must be recorded and analysed with regard to causality, seriousness and expectedness. A detailed protocol of the studies specifying the methods for collection, intervals for collection of the data and duration of follow up is requested.

4.4.1.3 Study in previously treated patients (PTPs)

Choice of patients

Previously treated patients (PTPs) with at least 150 treatment exposure-days to previous products are considered as low risk patients and should be evaluated for product related immunogenicity. These PTPs should be above 12 years of age, with a factor VIII level ≤1% and immunocompetent (CD4 lymphocytes >200/µl). The viral status of patients should be documented (HIV and HCV should be negative or have a viral load < 200 particles/µl). At least 50 frequently treated patients should be followed and documented for a minimum of 50 exposure days. These data should be provided with the application. Where patients are only rarely treated during a 6-month period (i.e. less than 10 total exposure days) they will not count towards the total number studied for immunogenicity, but should be included for other parameters of safety.

Immunogenicity testing

The factor VIII inhibitor titre should be determined by following the testing schedule set out in Annex III. In the clinical studies, it is proposed to perform sampling for inhibitor measurements not less than 3 days after the previous administration, if possible. For all patients who develop inhibitors a full clinical report should be provided including clinical relevance, the cumulative incidence and the number of exposure days. The titre of the inhibitor should be reported in Bethesda Units (BU) using the Nijmegen modification of the Bethesda assay.[1] Plasma samples of patients who are suspected of inhibitors or who have developed inhibitors should be stored for possible future testing. Reference is made to chapter 4.3.3.1.

4.4.1.4 Treatment of previously untreated patients (PUPs)

Previously untreated patients are defined as those patients who have never been treated with clotting factor products (except previous exposure to blood components). The product-related immunogenicity is more adequately addressed through studies of PTPs rather than PUPs. As stated in section 4.3.2, it is no longer considered appropriate to use clinical trials to investigate viral safety. For these reasons and since only a limited number of PUPs are available, there is no formal requirement for a PUP study to be carried out, but all treatment of PUPs and all adverse events should be documented. Experience with PUPs should be stated in the SPC.

Treatment in PUPs should not be initiated until data are available on 50 exposures for 20 patients (older than 12 years) who are included in the PTP trial.

1 Giles AR, Verbruggen B, Rivard GE, Teitel J, Walker I. A detailed comparison of the performance of the standard versus the Nijmegen modification of the Bethesda assay in detecting factor VIII:C inhibitors in the haemophilia A population of Canada. Association of Haemophilia Centre Directors of Canada. Factor VIII/IX Subcommittee of Scientific and Standardization Committee of International Society on Thrombosis and Haemostasis. Thromb-Haemost. 1998 Apr; 79(4): 872-5

4.4.1.5 Treatment of children

Since children may respond differently compared to adults, an open multicentre trial should include at least 20 children under the age of six years regardless of prior treatment but pre-treatment has to be documented. The clinical trial in children should not start before data are available on 50 exposures for 20 patients (older than 12 years) who are included in the PTP trial. The clinical trial should start with the investigation of pharmacokinetics. Factor VIII consumption (dose/kg for prophylaxis and therapy (on demand)) should be monitored as well as development of inhibitors in all the children participating in the study. Inhibitor testing should be performed following the same testing schedule as set out in Annex III or if there is any suspicion of inhibitor (see also 4.3.3.1). The study should continue until the patients have received a minimum of 50 exposures to the product. For all patients who develop inhibitors, a full clinical report should be provided including clinical relevance, the cumulative incidence and the number of exposure days in relation to development of inhibitors. The titre of the inhibitor should be reported in Bethesda Units, using the modified assay. Plasma samples from patients who are suspected of inhibitors should be stored for possible future testing.

Results of this study may be submitted after granting of a marketing authorisation but the study should have been started before. The number of children treated and/or the experience in children should be reflected in the SPC. The requirements of the paediatric regulation should be taken into account.

4.4.1.6 Post-marketing study

In view of the limited number of patients, data from pre-licensing studies are insufficient to estimate all aspects of therapy with FVIII. Therefore, to ensure consistency in the long-term between data from the clinical studies and from routine use, a post-marketing study has to be performed. The clinical study protocol has to be submitted with the application for marketing authorisation as part of the risk management plan (see Guideline on risk management systems for human use (EMEA/CHMP/96268/2005). The results of the PTP study should be taken into account for the design of the post-marketing study. Besides aspects like the general product safety and clinical efficacy, there has to be a focus on immunogenicity, particularly on inhibitor development and respective data. The general principles of immunogenicity and inhibitor documentation as laid down in chapter 4.3.3.1 should be taken into account.

The study should reflect the population in the countries the product is intended to be marketed. As a basic requirement, a detailed patient documentation (diary, logbook etc.) about the last 50 exposures/per patient in the last 2 years to confirm treatment modality (i.e. prophylaxis, on demand or recent surgery) is needed and should be available upon request. Patients with severe haemophilia after successful Immune Tolerance Induction (ITI) can be included, in order to obtain valuable information in this patient cohort. The proportion of these ITI patients should not be more than 25% of the whole cohort.

The number of patients to be enrolled for a post marketing study with a FVIII product should be a minimum of 200. In case inhibitors occur at an incidence of 1.5% or higher, with 200 patients there is at least 95% probability to observe antibodies in one or more patients.

An interim study report should be provided to competent authorities after 2 years of treatment, the study should be completed within 4 years.

For detailed requirements of study design see annex III.

4.5. CLINICAL TRIALS WITH NEW RECOMBINANT FACTOR IX PRODUCTS

4.5.1 Efficacy

A pharmacokinetic trial, should be performed in at least 12 subjects suffering from haemophilia B (factor IX ≤2%). The study should record incremental recovery, *in vivo* half-life, area under the curve (AUC), and clearance in patients without inhibitors who are not actively bleeding. Patients should be at least 12 years of age and should not have received an infusion of any FIX product for at least 4 days. Prior to the first administration of the factor IX product, half life of the previous product should be investigated in all patients. Samples for factor IX activity determination should be taken before

injection of 50-75 IU/kg of the new factor IX product and at 30 minutes, 1-3, 4-6, 7-9, 10-14, 20-26, 28-30, and 32-48 hours after the infusion. At least 3 different lots should be employed in the trial. Incremental recovery is determined as the peak level recorded 30 minutes after infusion and reported as [IU/ml]/[IU/kg]. As several methods are possible, the assay used should be described. Preferably the same assay should be used for analysis of the product and the patient's plasma.

It is very important to record the exact time post-infusion at which the actual samples were collected and to use these precise values in the analysis.

Patients taking part in the pharmacokinetic trial should continue treatment with the product for 6 months, and should be re-tested for the same pharmacokinetic parameters after 3-6 months using the same dose as in the first investigation.

Clinical efficacy of factor IX should be evaluated in at least 20 PTPs (>12 years), suffering from severe Haemophilia B (factor IX ≤ 2%, CD4 > 200/μL). The viral status of patients should be documented (HIV and HCV should be negative or have a viral load < 200 particles/μl). During an observation period of a minimum of 50 exposure days, clinical response should be assessed by the patients. Response should be assessed as "none", "moderate", "good" or "excellent" by the physician for those patients who were treated in hospital with the product for major bleeds. In addition, response will be determined by the physician in a minimum of 5 patients undergoing at least 10 surgical procedures, including efficacy of haemostasis, loss of blood, and requirements for transfusion.

For the assessment of clinical efficacy of factor IX claimed in long-term prophylaxis, patients should be followed for 6 months for bleeding episodes, bleeding intervals and number of treatments.

Clinical efficacy should be assessed by calculating the consumption of factor IX, expressed as number of infusions and IU/kg per month and per year, as well as IU/kg per event (prophylaxis, on-demand, and surgery).

Continuous infusion

If a claim for continuous infusion treatment is requested, clinical data are required to establish the efficacy and safety. A suggested protocol is described below.

The study should be carried out in at least 10 severe haemophilia B (FIX ≤2%) patients undergoing elective major surgical procedures.

Prior to surgery, a pharmacokinetic analysis in each individual should be performed to obtain, in particular, an estimate of clearance. The initial infusion rate could be based on the clearance as follows:

Clearance x desired steady state level = infusion rate (u/kg/hr)

(if necessary plus a corresponding safety margin)

After the initial 24 hours of continuous infusion, the clearance should be calculated again every day using the steady state equation with the measured level and the known rate of infusion.

Efficacy and safety data during surgery and for at least 6 days thereafter should be submitted, including PK parameters with the description of the assay used, daily dosage of factor IX with the description of the administration method used, administration rate, haemostatic response and blood loss, transfusion requirements and local and systemic side-effects.

Pharmaceutical data on reconstitution and stability of the product should be provided in the Quality section of the dossier.

4.5.2 Safety

In addition to the requirements for factor VIII products (see 4.4.1.2), appropriate tests for activation of coagulation (prothrombin fragment 1+2, thrombin-antithrombin (TAT) and D-dimer) should be carried out after administration of the product. This should be determined in the patients participating in the pharmacokinetic trial. Clinical evaluation of thrombosis should be undertaken by safe, objective means in a minimum of 5 patients undergoing at least 10 surgical procedures. .

In patients developing anaphylaxis and/or inhibitors to factor IX, data on relevant antibodies, e.g. IgE, IgG, against factor IX (using appropriate methods) should be submitted.

4.5.3 PTP study

Please refer to requirements for factor VIII products (see 4.4.1.3). Due to the lower incidence of haemophilia B as compared to haemophilia A, the number of previously treated patients followed up for immunogenicity may be lower than for factor VIII products: a minimum of 20 patients should be recruited.

4.5.4 Treatment of PUPs

See 4.4.1.4

4.5.5 Treatment of children

See 4.4.1.5

Due to the lower incidence of haemophilia B as compared to haemophilia A, the number of previously treated patients followed up for immunogenicity may be lower than for factor VIII products: 12 patients.

4.5.6 Post-marketing study

The number of patients to be enrolled for a post marketing study with FIX product should be a minimum of 50.

General principles of study performance: see section 4.4.1.6

4.6. CHANGE IN THE MANUFACTURING PROCESS OF AUTHORISED PRODUCTS

4.6.1 Introduction

Changes in the manufacturing procedures may lead to significant changes in the product and may thereby alter the structure of the coagulation factor and its activity. The effects of changes in the manufacturing process (e.g. new purification procedures and/or omitting human or animal-derived proteins during manufacture) on the biological characteristics and activity of the product should be investigated. If significant impact on the activity of the coagulation factor cannot be excluded, data on pharmacokinetics, efficacy and safety should also be provided with the application.

Two inhibitor outbreaks occurred in the early 1990's in previously tolerant patients who had been treated for a number of years following exposure to a plasma-derived factor VIII product subjected to a modified virus inactivation method. Hence the incidence of inhibitor formation may be affected by the type of product used for treatment and its potential to result in alteration of factor VIII molecules, 'neoantigens'. Such inhibitors could be demonstrable in previously treated patients.

4.6.2 Clinical trials with human recombinant factor VIII products

4.6.2.1 Efficacy

Evidence should be provided to demonstrate that the change in the manufacturing process has not affected the pharmacokinetics of the product.

A comparative pharmacokinetic trial with pre-change product versus the post-change product should be performed in at least 12 subjects suffering from haemophilia A (factor VIII ≤1%). The study should record incremental recovery, *in-vivo* half-life, area under the curve (AUC), and clearance in patients without inhibitors who are not actively bleeding. Patients should be at least 12 years of age and should not have received an infusion of any FVIII product for at least 4 days. Samples for factor VIII activity determination should be taken before injection of 25-50 IU/kg of the factor VIII product and at 30 minutes, 1-3, 4-6, 7-9, 10-14, 20-26, 28-30 and 32-48 hours after the infusion. At least 3 different lots of the post-change product should be employed in the trial. Incremental recovery is determined as the peak level recorded within the three hours after infusion and reported as [IU/ml]/[IU/kg].

It is anticipated that some deviation from the recommendation may occur in clinical practice. For this reason, it is very important to record the exact time post-infusion at which the actual samples were collected and to use these precise values in the analysis.

Patients taking part in the pharmacokinetic trial should continue treatment with the post-change product for 6 months, and should be re-tested for the same pharmacokinetic parameters after 3-6 months using the same dose as in the first investigation.

Should any of the patients participating in the clinical trials undergo surgical procedures, response will be determined by the physician, including efficacy of haemostasis, loss of blood and requirement for transfusion and occurrence of thromboembolic episodes.

4.6.2.2 Safety

Please refer to requirements for new human recombinant factor VIII products. (See 4.4.1.2).

4.6.2.3 PTP study

See 4.4.1.3

4.6.2.4 Post-marketing study

See 4.4.1.6

4.7 CLINICAL TRIALS WITH RECOMBINANT FACTOR IX PRODUCTS

4.7.1 Efficacy

Evidence should be provided to demonstrate that the change in the manufacturing process has not affected the pharmacokinetics of the product.

A comparative pharmacokinetic trial with the pre-change product versus the post-change product should be performed in at least 12 subjects suffering from haemophilia B (factor IX ≤2%). The study should record incremental recovery, *in-vivo* half-life, area under the curve (AUC), and clearance in patients without inhibitors who are not actively bleeding. Patients should be at least 12 years of age and should not have received an infusion of any FIX product for at least 4 days. Samples for factor IX activity determination should be taken before injection of 50-75 IU/kg of the new factor IX product and at 30 minutes, 1-3, 4-6, 7-9, 10-14, 20-26, 28-30, and 32-48 hours after the infusion. At least 3 different lots of post-change product should be employed in the trial. Incremental recovery is determined as the peak level recorded within the three hours after infusion and reported as [IU/ml]/[IU/kg].

It is anticipated that some deviation from the recommendation may occur in clinical practice. For this reason, it is very important to record the exact time post-infusion at which the actual samples were collected and to use these precise values in the analysis.

Patients taking part in the pharmacokinetic trial should continue treatment with the post-change product for 6 months, and should be re-tested for the same pharmacokinetic parameters after 3-6 months using the same dose as in the first investigation.

Should any of the patients participating in the clinical trials undergo surgical procedures, response will be determined by the physician, including efficacy of haemostasis, loss of blood, requirement for transfusion and occurrence of thromboembolic episodes.

4.7.2 Safety

In addition to the requirements for factor VIII products (see 4.4.1.2), appropriate tests for activation of coagulation (prothrombin fragment 1+2, thrombin-antithrombin (TAT) and D-dimer) should be carried out after administration of the product. This should be determined in the patients participating in the pharmacokinetic trial. Clinical evaluation of suspected incidences of thrombosis should be undertaken by safe, objective means in any patients undergoing surgical procedures.

In patients developing anaphylaxis and/or inhibitors to factor IX, data on relevant antibodies, e.g. IgE, IgG against factor IX (using appropriate methods) should be submitted.

4.7.3 PTP study

See 4.4.1.3 and 4.5.3

4.7.4 Post-marketing study

The number of patients to be enrolled for a post marketing study with FIX product should be a minimum of 50.

General principles of study performance: see section 4.4.1.6.

422 **ANNEX I**

423 **Clinical trials with recombinant factor VIII products: new products**

424

TRIAL, SUBJECTS	INVESTIGATION	PARAMETERS
12 haemophilia A patients (factor VIII ≤1%) without inhibitors and not actively bleeding.	1. Pharmacokinetics	Incremental recovery, half-life*, AUC, clearance. Patients should be re-tested after 3-6 months (including F VIII inhibitor assay).
	2. Safety	Blood pressure, heart rate, temperature, respiratory rate and adverse events.
5 haemophilia A patients undergoing at least 10 surgical procedures.	1. Clinical efficacy	Efficacy of haemostasis, loss of blood and requirement for transfusion. Factor VIII consumption.
	2. Safety	Adverse events.
PTP study 50 PTPs (>12 years) (factor VIII ≤1% and CD4>200/µl).	1. Clinical efficacy	Factor VIII consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor titre in Bethesda Units, using the Nijmegen modification of Bethesda assay, immediately before first exposure, ED1, ED 10-15, ED 50-75 or if there is any suspicion of inhibitor development, continue for a minimum of 50 exposure days.
	3. Safety	Adverse events.
Treatment of PUPs.	All treatment of PUPs should be documented.	
Open multicentre trial in 20 children with haemophilia A (<6 years) to be started after results of 50 exposures in 20 PTPs (>12 years). Beginning of patient enrolment before marketing authorisation.	1. Clinical efficacy	Factor VIII consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor testing immediately before first exposure, ED1, ED 10-15, ED 50 or if there is any suspicion of inhibitor development. Continue until a minimum of 50 exposure days.
	3. Safety	Adverse events
Post-marketing study.	1. Clinical efficacy	Protocol should be provided according to annex III.
	2. Immunogenicity	
	3. Safety	

425 *half-life should also be measured with the previous product.

426 **ANNEX II**

427 **Clinical trials with recombinant factor VIII products following changes of manufacturing**
428 **process**

429

TRIAL, SUBJECTS	INVESTIGATION	PARAMETERS
12 haemophilia A patients (factor VIII ≤1%) without inhibitors and not actively bleeding.	1. Pharmacokinetics	Comparative trial pre-change vs post-change product: incremental recovery, half-life, AUC, clearance. Patients should be tested again after 3-6 months (including F VIII inhibitor assay).
	2. Safety	Blood pressure, heart rate, temperature, respiratory rate and adverse events.
Any haemophilia A patients undergoing surgical procedures.	1. Clinical efficacy	Efficacy of haemostasis, loss of blood and requirement for transfusion. Factor VIII consumption.
	2. Safety	Adverse events
PTP study 50 PTPs (>12 years) (factor VIII ≤1% and CD4>200/µl).	1. Clinical efficacy	Factor VIII consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor titre in Bethesda Units, using the modified assay, immediately before first exposure, ED1, ED 10-15, ED 50 or if there is any suspicion of inhibitor development. Continue until a minimum of 50 exposure days
	3. Safety	Adverse events.
Post-marketing study.	1. Clinical efficacy	Protocol should be provided according to annex III.
	2. Immunogenicity	
	3. Safety	

ANNEX III

Requirements for PMS study

Inclusion criteria

- Diagnosis; haemophilia A
- Severity: < 0.01 IU/ml i.e < 1% factor VIII:C
- Number of exposure days before inclusion: > 150 exposure days
- Age: > 12 years

Documentation of Patient's characteristics

- Gene defect
- Ethnicity
- Family history for haemophilia
- History for inhibitors
- Viral status

  (HIV and HCV should be negative or have a viral load < 200 particles/µl.)
- Co-morbidity or co-medication which would significantly impact blood coagulation or immunoreaction (any information concerning this issue should be included)

Patient enrolment

- At least 200 patients per PMS study*
- Duration / Follow up = at least 50 ED

* progress on recruitment has to be reported on a regular basis (will be set out before approval of procedure)

General performance

- Before patient inclusion there should not be a clinical suspicion for an inhibitor; and a recovery and inhibitor test in a central laboratory should confirm that the patient is inhibitor negative at study entry. An inhibitor test which is not negative should be confirmed by testing a 2nd separately drawn sample in a central laboratory.

- Testing schedule (ED=Exposure Day)

	Previous product * #	Test product ED1*	Test product ED10-15*	Test product ED50-75*
Inhibitor	x	x	x	x
Recovery	x	x	x	x

*after washout period (see Explanatory Note); storage of second back up blood sample is recommended

New patients= not recruited for pre-authorisation studies

- Patients' diaries should be evaluated on total number of exposures per year and mean dose per kg per patient/ year (consumption).
- Intended treatment regimen for every patient at study entry and reason for each ED should be documented
- In case of bleedings:

  documentation of particulars; judgement of severity and treatment outcome by clinician and patient
- In case of surgery different data are to be collected (surgical protocol)

  (e.g. type of surgery (planned or emergency); documentation of complications; mode of administration, consumption)

Explanatory Note

Inhibitor tests should be performed when the plasma FVIII level has reached a pre-substitution nadir (documentation for the last infusion should be provided). In the case that patients are treated on demand, an inhibitor can be missed when the patients did not receive treatment for > 2 weeks. According to the t1/2 of immunoglobulins, the inhibitor will drop gradually when treatment has been stopped. In case of a positive inhibitor test, also PK/ recovery tests are necessary to confirm inhibitory activity.

Co-medication: At the present time, all patients are accepted in studies. Patients with HIV infection receive intensive co-medication, HAART therapy, it is unknown whether this can influence inhibitor formation or efficacy of treatment. Similar problems can be expected for HCV positive patients, some receive therapy and others have lower platelets and decreased liver function and altered coagulation. Probably these patients can be included to have more data on efficacy in this group but more parameters on co-morbidity have to be collected.

ANNEX IV

Clinical trials with recombinant coagulation factor IX products: <u>new products</u>

TRIAL, SUBJECTS	INVESTIGATION	PARAMETERS
12 haemophilia B patients (factor IX ≤2%) without inhibitors and not actively bleeding.	1. Pharmacokinetics	Incremental recovery, half-life*, AUC, clearance; Patients should be re-tested after 3-6 months.
	2. Safety	Blood pressure, heart rate, temperature, respiratory rate and adverse events. Thrombogenicity.
5 haemophilia B patients undergoing at least 10 surgical procedures.	1. Clinical efficacy	Efficacy of haemostasis, loss of blood and requirement for transfusion. Factor IX consumption.
	2. Safety	Adverse events, Thrombogenicity.
PTP study 20 PTPs (>12 years) (factor IX ≤2% and CD4>200/µl).	1. Clinical efficacy	Factor IX consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor titre in Bethesda Units immediately before first exposure, ED1, ED 10-15, ED 50-75 and if there is any suspicion of inhibitor development; Follow up for a minimum of 50 exposure days.
	3. Safety	Adverse events.
Treatment of PUPs.	All treatment of PUPs should be documented.	
Open multicentre trial in 12 children with haemophilia B (<6 years) to be started after results of 50 exposures in 20 PTPs (>12 years) have become available but before marketing authorisation.	1. Clinical efficacy	Factor IX consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor titre in Bethesda Units immediately before first exposure, ED1, ED 10-15, ED 50-75 and if there is any suspicion of inhibitor development. Follow up for a minimum of 50 exposure days.
	3. Safety	Adverse events.
Post-marketing study	1. Clinical efficacy	Protocol should be provided
	2. Immunogenicity	
	3. Safety	

*half-life should also be measured with the previous product.

ANNEX V

Clinical trials with recombinant coagulation factor IX products: <u>following changes of manufacturing process</u>

TRIAL, SUBJECTS	INVESTIGATION	PARAMETERS
12 haemophilia B patients (factor IX ≤2%) without inhibitors and not actively bleeding.	1. Pharmacokinetics	Comparative trial pre-change product vs. post-change product: incremental recovery, half-life, AUC, clearance. Patients should be tested again after 3-6 months
	2. Safety	Blood pressure, heart rate, temperature, respiratory rate and adverse events. Thrombogenicity.
Any haemophilia B patients undergoing surgical procedures.	1. Clinical efficacy	Efficacy of haemostasis, loss of blood and requirement for transfusion. Factor IX consumption.
	2. Safety	Adverse events. Thrombogenicity.
PTP study. 20 PTPs (>12 years) (factor IX ≤2% and CD4>200/µl).	1. Clinical efficacy	Factor IX consumption, physician's assessment of response in treatment of major bleeds.
	2. Immunogenicity	Inhibitor titre in Bethesda Units immediately before first exposure, ED1, ED 10-15, ED 50- 75 and if there is any suspicion of inhibitor development; Follow up for a minimum of 50 exposure days.
	3. Safety	Adverse events.
Post-marketing study.	1. Clinical efficacy	Protocol should be provided.
	2. Immunogenicity	
	3. Safety	