SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Ceprotin. This scientific discussion has been updated until 1 June 2002. For information on changes after this date please refer to module 8B

1. Introduction

CEPROTIN is a plasma-derived protein C concentrates. CEPROTIN is indicated in purpura fulminans and coumarin induced skin necrosis in patients with severe congenital protein C deficiency. Furthermore CEPROTIN is indicated for short-term prophylaxis in patients with severe congenital protein C deficiency if one or more of the following conditions are met:

- surgery or invasive therapy is imminent
- while initiating coumarin therapy
- when coumarin therapy alone is not sufficient
- when coumarin therapy is not feasible.

Protein C (PC) is synthesised in the liver as a vitamin K-dependent plasma protein. PC is a 62,000 molecular weight glycoprotein that circulates in the blood as an inactive zymogen. Thrombin rapidly activates PC after binding to thrombomodulin, a membrane receptor protein found on endothelial cells. The PC pathway provides a natural mechanism to control the coagulation system and to prevent excessive clotting. In individuals with a deficiency of PC the defect in the control mechanism leads to increased coagulation activation, resulting in thrombin generation and ultimately intravascular clot formation with thrombosis.

An inherited PC deficiency is autosomal in nature and associated with an increased risk of venous thrombosis. Phenotypically two types have been described: type 1 in which both antigenic and functional levels are reduced and type 2 in which functional levels are reduced to a greater extent than antigenic levels. There also appear to be both dominant and recessive forms of PC deficiency. Heterozygous, clinically "overt" PC deficiency (dominant) has a prevalence of 1/16,000-1/36,000 in the general population (*Cooper & Tuddenahm , Br Med Bull 1994; 50:833-50*). An asymptomatic (recessive) "covered" form in which PC activity levels are consistent with a heritable heterozygous deficiency state may occur in up to 1/200 of the general population (*Miletich et al., N Engl J Med 1987; 317:991-6*). Homozygous type 1 PC deficiency is rare, with only 17 cases recorded in an international data base of mutations (*Reitsma et al., Thromb Haemost 1995; 73:876-9*) and only about 20 further cases reported in the literature (*Baliga et al., Eur J Pediatr 1995; 154: 534-8*). Most commonly homozygotes present with purpura fulminans in the neonatal period. These infants have PC levels less than 0.10U/ml. A small number of severe PC deficiencies do not present purpura fulminans at birth, but contract severe (venous) thromboembolic disease during childhood or early adult life. PC levels in these patients are usually measurable and range from 0.02 to 0.23 U/ml.

There are two main clinical features of severe PC deficiency: purpura fulminans in homozygous newborns and skin necrosis when oral anticoagulant therapy is started. This latter complication is caused by a temporary procoagulant imbalance in the early phase of use of vitamin K antagonists. The pro-thrombotic state is caused by the persistence of high circulating levels of procoagulant vitamin K-dependent factors with a long half-life, which are no longer opposed by the rapidly falling levels of PC with a short half-life.
The clinical signs of purpura fulminans are the result of capillary thrombosis and interstitial bleeding and consist of ecchymotic skin lesions that, if untreated, rapidly develop into haemorrhagic bullae with subsequent gangrenous necrosis, which may necessitate amputation. These lesions appear mainly on the extremities but also on the buttocks, abdomen, scrotum, and scalp. Thrombosis may occur in larger vessels and multi-organ failure develops, with severe disseminated intravascular coagulation (DIC). If the condition is left untreated, the progression of the thrombotic lesions results in blindness, severe brain damage, multi-organ failure, and death.

Though requiring intravenous access and frequent infusions due to PC's short half-life, replacement therapy appears the obvious approach in the acute manifestations of the disease and in the short-term secondary prevention of thromboembolic episodes. Fresh frozen plasma (FFP) has been used, but the development of hyperproteinemia, hypertension, loss of venous access and the risk of exposure to viral agents limit its administration on a frequent basis. Better results are expected with PC concentrates.

CEPROTIN is a stable, monoclonal antibody purified concentrate of PC zymogen. CEPROTIN is manufactured from pooled human plasma by means of immunoaffinity chromatography using an immobilized murine monoclonal antibody. Two independent viral inactivation procedures are incorporated in the manufacturing process.
The product is supplied as a freeze-dried substance in single dose vials together with the necessary amount of sterilised water for injection. The reconstituted solution is intended for intravenous injection and is presented in two sizes, 500 and 1000 IU.

The Applicant did not at first intend to develop the product commercially. A program of pre-clinical testing was carried out to investigate the safety of the product. However, in response to requests from physicians, the Applicant began supplying the product for compassionate use. The Applicant argues that it was impossible to organise prospective, large-scale, controlled clinical trials, since:

- Homozygous or double heterozygous PC deficiency is extremely rare;
- Most subjects are severely ill at the time of diagnosis;
- The small patient group is comprised primarily of small children and babies;
- Treatment options are limited;
- The need to measure PC levels for dosing would interfere with any blinding of study medication.

Since the efficacy/safety profile of this product has not been fully established yet, its use is deemed relatively safe and effective only in the severe clinical conditions for which it is indicated. The applicant for this medicinal product is BAXTER AG, Austria.

2. Chemical, pharmaceutical and biological aspects

Composition

The product is supplied as a freeze-dried substance in single dose vials together with the necessary amount of sterilised water for injection. The reconstituted solution is intended for intravenous injection and is presented as, 500 and 1000 IU. The proposed composition of the medicinal product is as follows:

	Unit and/or percentage formula		Function	Reference to standard
I Active Ingredient				
Protein C	500 IU	1000 IU	Anticoagulatory proenzyme	WHO
Total Protein	42,5 mg	85 mg	Active Ingredient and stabilizer	
Thereof: Human Albumin	40 mg	80 mg	Stabilizer	Ph.Eur.

Excipients				
Sodium Chloride	44 mg	88 mg	Achieving isotonia	Ph.Eur.
Trisodium Citrate dihydrate	22 mg	44 mg	Stabilizer	Ph.Eur.
II Vial containing Solvent				
Sterilized water for injections	5 ml	10 ml	Solvent	Ph.Eur.

The product is filled either with nominally 500 IU into vials of neutral glass of hydrolytic type I or with nominally 1000 IU into vials of surface-treated soda lime silica glass of hydrolytic type II. The vials are closed with stoppers of chlorobutyl rubber. Both vials and stoppers comply with the respective Ph. Eur. requirements.

CEPROTIN is to be dissolved in 5 ml and 10 ml sterilised water for injections, respectively.

Active substance

Source material
The source material for the production of PC Bulk Solution is human plasma complying with the requirements of the monograph "Human Plasma for Fractionation" (Ph. Eur.: 0853). 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 "Contribution to Part II of the structure of the dossier for applications for marketing authorization – Control of starting materials for the production of blood derivatives".
General information has been provided on remuneration of donors, number of donation centres, internal audits as well as audits performed by Baxter and inspections by competent authorities.
Donor selection criteria described are in accordance with WHO and Council of Europe recommendations as well as with national regulations.
Plasma is collected in Austria, Czech Republic, Germany, Sweden and USA. Names and addresses of the plasma suppliers are provided. Plasmapheresis and/or transfusion services are controlled and inspected by competent authorities. Baxter's personnel audit plasma suppliers at least once in 24 months.

The epidemiological data of blood-borne infections are presented in very clear and detailed summary tables.
Assurance is given that centres supplying plasma to the applicant do not collect from populations at high risk for blood-borne infections. The applicant has provided the epidemiological data on plasma collected at the donation centres in Europe and USA. Every individual plasma donation is tested for the absence of HBsAg, of HIV-1/2 Ab, and HCV Ab by using officially approved test kits. Any donation not meeting the test requirements is discarded. A list of the test kits used for screening the donation is provided. The applicant notes that plasma suppliers agree in the Standard Contract to use only test kits, which are FDA or PEI, licensed or licensed by the health authorities in France or UK. Moreover, all laboratories routinely take part in proficiency studies to prove and assure accuracy of the results. The successful participation in such proficiency programs has to be shown to the applicant annually by each laboratory.
The applicant states that in addition to current recommendations and regulations for plasma collection, a Plasma Safety Program has been introduced. The main features of this Program are the donor applicant quarantine program and the inventory hold of donations.

The applicant states its ability to trace the path of any plasma donation to the final product and *vice versa*. It is mentioned that the applicant will be notified of any defective plasma by the donation centre through a Reporting Program. Moreover, the criteria governing product recall has also been provided.

Each plasma pool is tested for the absence of HBsAg, HIV-1/2 Ab and HCV Ab by ELISA. For this purpose, only test kits approved by FDA or PEI are used. The validation reports have been provided.

All plasma pools are also tested for viral nucleic acids of HIV-1/2, HBV and HCV by a PCR test, which was set up and validated by the applicant. The validation of the NAT systems covers the main relevant points with satisfactory results. The implementation of the HCV NAT is in compliance with the European regulation (CPMP/BWP/390/97). The results of the validation studies meet the requirements of the European OMCL Network guideline. The protein content and the bioburden of plasma pools are routinely monitored.

A list of blood bags and plasma pooling bottles, with information on anticoagulants and CE marking, is provided as recommended by CPMP document III/5272/94.

Assurance is given that for all plasma shipments to the applicant, the temperature limits for storage and for plasma transport comply with the Ph. Eur. monograph "Human Plasma for Fractionation".
The average storage time for plasma is 6 months from the date of donation; the maximum storage time is 3 years.
The standard contract between the Manufacturer and plasma collection establishments has been attached. Verification that the contract is applied in all centres has been provided and a commitment that all plasma suppliers have confirmed the contents of the contract has been included in the dossier.

Purification

Process validation
Protein C concentrate is manufactured essentially by chromatographic procedures including immunoaffinity chromatography on solid phase monoclonal antibody. Cleaning of the columns was properly validated. The production process leading to a PC-enriched fraction and the subsequent purification steps leading to the production of protein C concentrate bulks are described.
The flow charts are included in the dossier submitted.
Briefly, cryosupernatant is obtained from frozen plasma and applied to an ion exchange gel. After extensive washing, the matrix is eluted and the eluate is submitted to an ultrafiltration step. The ultrafiltrate is then treated with Polysorbate 80, diluted and applied onto an ion exchange gel column. PC–enriched fraction is eluted and submitted to further purification steps. The applicant states that these initial manufacturing steps are identical to those used for the production of IMMUNINE, a high-purity Factor IX Concentrate manufactured by Baxter AG.
The main purification step is an immunoaffinity chromatography using immobilised murine monoclonal antibody against PC. Further ion exchange chromatography steps are implemented to remove any murine IgG as well as other contaminants. After addition of human albumin as stabilizer, the bulk is subjected to a two-step vapour heat treatment for virus inactivation, followed by ion exchange chromatography.

The Applicant provides a list of the in-process controls performed at each step of production of the active ingredient to assure compliance with defined operating parameters. In addition, process parameters related to product quality, such as purity, specific activity, are monitored throughout the process.

Specifications were defined for the intermediates to ensure consistent quality of the product.

The release test program for PC Bulk Solution includes the following tests: PC, protein content, specific PC activity, activated PC, murine IgG and Polysorbate 80. Current analytical results have been provided.
A list of the major equipment used in the manufacturing steps performed is provided.

Rooms are mainly classified according to European GMP requirements.

Specifications of the active substance
The specifications for the bulk material have been set and are appended to this assessment report.

They are based on total protein content, PC activity, specific activity, activated PC activity, sodium chloride and trisodium citrate-dihydrate content, pyrogens and mouse IgG content. Data on five batches are given.

Batch to batch consistency
The validation data provided give consistency of the production steps and the suitability of the selected in-process controls.

Other ingredients

Most of the excipients and auxiliaries used for the production of PC Bulk Solution, including those used only in the purification process, comply with pharmacopoeial requirements. Only the Antithrombin III-heparin complex, the ion exchange gels and a few reagents are not described in a pharmacopoeia.

For the non-pharmacopoeial reagents, only high-purity or analytical grade chemicals are used, which are supplied by the manufacturer with guaranteed specifications and batch-specific certificates of analyses.

The various chromatography gels have well-defined structures and are especially suited for the separation of proteins. In addition to the certificates of analysis submitted by the respective manufacturer, the applicant tests them for gel capacity and/or separation performance.

Antithrombin III-heparin complex is a freeze-dried concentrate, prepared by Baxter AG by a patented process. For virus inactivation, the intermediate product (antithrombin III) is subjected to a heat treatment in aqueous solution. The applicant states that the starting plasma comply with the same requirements as the source material for PC Bulk Solution. In–house specifications have been established for the antithrombin III-heparin complex in accordance with the pharmacopoeial requirements for blood products. A dossier concerning antithrombin III has been provided with the responses of the company to the CPMP list of questions including information on the composition, method of preparation, control of starting materials, control test on intermediate products, control test on the finished product and stability data. Antithrombin III used during production is still in its shelf life at the end of the manufacturing process of CEPROTIN.

Human albumin, sodium chloride, and sodium citrate•$2H_2O$ are used as excipients in the pharmaceutical formulation of CEPROTIN. The applicant indicates that all of them are in compliance with the relevant monographs of European Pharmacopoeia. Baxter AG manufactures human albumin from human plasma that complies with the same requirements that apply to production of PC Bulk Solution. The manufacturing method of human albumin includes a virus inactivation. A dossier concerning albumin has been provided with the responses of the company to the CPMP list of questions including information on the composition, method of preparation, control of starting materials, control test on intermediate products, control test on the finished product and stability data. Albumin used during production is still in its shelf life at the end of the manufacturing process of CEPROTIN and the expiry date of albumin used, as excipient is not earlier than that of the finished product.

Product development and finished product

Product development
The applicant indicates that, during the development of the product, modifications were made to the original manufacturing process (method I). This method included two virus-inactivation steps based on vapour heat treatment while in the current manufacturing process (method II) the first treatment employs Polysorbate 80 and the second one is a vapour heat treatment. In addition, method II includes five more ion-exchange chromatography steps. In both methods the purification of the product was performed with the immobilised monoclonal antibody. However, in method I mAb was mouse ascites derived, whereas in the current method mAb is cell culture derived.

Clinical trial formula
Some of the earlier clinical investigations were performed using protein C concentrate manufactured by method I. The pharmaceutical documentation only refers to method II. The applicant data allow comparison that the final product release specifications are the same for both products.

Manufacturing process
The whole process is performed in Baxter AG, Industriestrasse 131, Benatzkygasse 2-6, Industriestrasse 72 and Smolagasse, Vienna, Austria. A clear flow sheet of the manufacturing process has been provided.
In-process controls (IPCs) and acceptance criteria as well as test methods are given. The IPCs appear to control the process appropriately.

For the formulation of protein C concentrate, vapour heated PC bulk solution is used. One batch of vapour-heated solution (3-12 kg) is manufactured from a total of 10,000 – 40,000 litres of plasma, processed as 1,600 – 1,000 litres pools.
Flow chart of the whole manufacturing process is provided in the dossier.

Starting with 6 kg vapour heated bulk solution the final bulk solution has the following composition:

Protein C	105,000 IU/L
Human albumin	8 g/L
Sodium chloride	8.8 g/L
Sodium citrate	4.4 g/L

The pH is adjusted to 7.0 ± 0.1 by addition of 2% hydrochloric acid or 2% sodium hydroxide solution. After sterile filtration the final bulk solution is filled in 12 ml vials of 500 or 1000 IU/vials.

Cleaning and treatment of glass vials and rubber stoppers are described. The product is then freeze-dried. The freeze-drying process is described and validation provided.
In process controls for all steps are clearly reported and acceptance criteria are given.
The applicant indicates that GMP are always followed.

Finished product specification
The finished product specification is appended to this assessment report.
The test program and the specifications for the final product have been defined in analogy to pharmacopoeial requirements for other human coagulation factor concentrates. They are considered adequate and they include identity, potency, excipients and safety of the preparation as well as general pharmaceutical tests.
The results of three batches of each filling size show consistency of the manufacturing process and full compliance with the product specifications.

Stability data
Stability of intermediates
Stability of intermediates has been proven. However, equivalence of the containers used in production and in stability studies has still to be provided.

Finished product stability
Based on the results provided on 3 batches of each filling size, the data support the proposed shelf-life of two years when stored cool between +2 °C and +8 °C.

Stability reconstituted product
According to the SPC the product should be administered immediately. Stability data after reconstitution allow the solution to stand for 3 hour at room temperature.

Immunoaffinity matrix

Monoclonal antibody
A selective immune affinity chromatography step is included in the purification process of CEPROTIN. Development, characterisation, fermentation and purification of this monoclonal antibody are described in sufficient detail.
The column is coupled with a monoclonal antibody specific for Protein C. This antibody, was obtained by fusion of myeloma cell line with splenocytes from mice immunised with purified Protein C. Mice were routinely tested every six months for Group I and Group II viruses. Cell banks were prepared by a contract manufacturer, Polymun Scientific, Vienna, and mainly characterised in a contract laboratories, Q1 Biotech Ltd, Glasgow, Scotland.
Production of mAb was performed on a campaign in 1992/93 at pilot scale and 1996 upscaled. Due to changes introduced between the two campaigns, only the two batches produced in 1996 were used for validation purposes. Data submitted by the applicant support the comparability of the mAb obtained with the two processes.
The cell bank system has been fully characterised for the presence of viruses and adventitious agents. For production of mAb, a repeated batch system is used up to a total fermentation volume of 500 L. A Post Production Cell Bank was also established and characterised.
The manufacture of the monoclonal antibody is sufficiently described.

Compliance with TSE guideline
As mentioned in the TSE guideline, MCB are exempted from the requirements for Certification of suitability of monographs of the Eur. Ph.
Currently, no more monoclonal antibody is available where FCS from Salzman Inc., Iowa, USA, was used to establish the MWCB, for which a Certificate of suitability cannot be provided. Furthermore, no more CEPROTIN lots are available which have been manufactured using this monoclonal antibody.
For the establishment of the MWCB for the production of the next monoclonal antibody lot, the company will use FCS from Hyclone Laboratories INC, Utah, and USA. For which a certificate of suitability is available.
The manufacturing process will fully comply with all relevant guidelines regarding TSE risk.

Furthermore, in order increase the overall assurance of safety of CEPROTIN with respect to TSE risk, the applicant has the intention to submit a type II variation to introduce a change in the manufacturing process of the mAb used in the immunoaffinity chromatography purification step of CEPROTIN. At present the applicant has submitted an application for scientific advice on whether the Company's investigation program and data base outlined above would be sufficient to show the equivalence of the monoclonal antibody produced by the current process and the new proposed, where the MWCB, to be used in the manufacture of the future mAb batches is planned to be stored in protein free medium.

Viral safety and safety with respect to TSEs

Five steps of the manufacturing process of Protein C were evaluated for their capacity for virus removal/inactivation: Polysorbate 80 treatment, anion exchange chromatography I (non binding mode), anion exchange chromatography II (binding mode), immune affinity chromatography and the vapour heat treatment. Virus validation studies were performed with HIV-1 and hepatitis-A-Virus (HAV) as well as the model viruses for hepatitis C Virus (HCV) (Bovine Viral Diarrhoea Virus (BVDV) and Tick Borne Encephalitis Virus (TBEV)), the model for human herpes viruses (Pseudorabies Virus (PRV)) and a model for the human parvovirus B19 (Minute Mice Virus (MMV)). The selection of the viruses corresponds with the requirements (CPMP/BWP/269/95). The results of the virus validation studies are summarised in the table.
The manufacturing process of Protein C contains several steps, which contribute to the virus safety: the heat treatment, the treatment with Polysorbate80 and the immune affinity chromatography.

Table: Results of the virus validation studies

	HIV-1		**BVDV/TBEV**		**PRV**		**HAV**		**MVM**	
	Reduction factor [log10]									
	runA	runB	runA	runB	runA	runB	runA	runB	runA	runB
Polysorbate 80 treatment	>6.2	>6.4	>3.8	>4.8	<1.0	<1.0	<1.1	<1.0	<1.0	<1.1
Ion exchange chromatography (non binding mode)(*)	n.a.	n.a.	n.a.	n.a.	1.4	<1.0	n.a	n.a.	1.3	<1.0
Ion exchange chromatography (binding mode)(**)	>5.7		>7.3		<1.0		2.6		1.3	
Immune affinity chromatography	>5.7	5.7	3.3	6.2	4.6	4.9	3.1	3.4	2.2	2.6
Vapour heat treatment	>6.5	>6.0	>5.2	>5.3	>7.2	>7.0	>6.0	>6.0	1.5	<1.0

(*)Ion exchange chromatography I seems to be ineffective for virus removal.
(**)The validation report for the ion exchange chromatography II is incomplete and insufficient. As a result, the reduction factors for this step which are listed in the table cannot be accepted.

The most important step for the virus safety of Protein C is the two step vapour heat treatment (10 h at 60°C and 1 h at 80°C): HIV-1, BVDV, PRV and HAV were inactivated below the detection limit. Parvovirus, MMV, was not inactivated.
The Polysorbate 80 treatment is effective for HIV-1 and BVDV but cannot inactivate PRV. However, the value of this step for other viruses must not be presumed from the HIV-1 and BVDV data and has to be clarified individually for each virus. Consistency of the Polysorbate 80 concentration has been demonstrated by analytical data of 10 production batches. Data demonstrate a sufficient high level of virus inactivation during the sanitisation procedure using 0.2 M NaOH.
The immune affinity chromatography has some effectiveness for removal of HIV-1, TBEV, PRV and HAV. Reduction factors between >5.7 $\log_{10}$ and 3.1 $\log_{10}$ have been reported. However, the company should complete the down scaling report and investigate this procedure further in order that the reliability of the above listed reduction factors can be confirmed; especially the partitioning of HIV-1 and the differences in the removal of TBEV in the two runs should be clarified.
The sanitisation of the immune affinity column is performed by treatment with 0.75 M Guanidine.HCl as the antibody is sensitive to NaOH-treatment. The virus data demonstrate that viruses cannot completely be inactivated and viruses may in principle accumulate on the column. However, as the antibody is sensitive against a more robust column regeneration process, a change in the sanitisation process will not be required. The company should be aware of the risk of virus accumulation in the column and should consider this in setting limits for the re-use of the column. The applicant will investigate the robustness of virus removal by the immune affinity chromatography and a commitment has been given to submit the study results.
According to the virus validation reports, the process has no capacity for removal/inactivation of parvovirus. In order to avoid Protein C contamination with Parvovirus B19, the exclusion of viremic high-titre from the manufacturing process of CEPROTIN, identified by PCR screening, would be appropriate to improve the viral safety of CEPROTIN with regard B19. However, this is a general problem of many coagulation factor concentrates on the market.
Summarising, the existing documentation provide reasonable confidence that the viral safety is comparable with other products, which are on the market.

Safety assessment of other components used for manufacture
The process contains some additives, human albumin, human antithrombin III and porcine heparin.
The antithrombin III is used so early in the Protein C manufacturing process that inactivation/removal stages for Protein C also apply to it. There is no concern about the use of antithrombin III for the manufacture of Protein C.
Albumin is manufactured according to the "classical" ethanol fractionation and pasteurised, 60°C for 10 h, as required by the Ph Eur. The safety of albumin corresponds therefore with other products on the market and there are no concerns about its use for Protein C.
The company has described the manufacturing process and provided an appropriate risk assessment for heparin.

For the immune affinity chromatography the monoclonal antibody is used. The antibody is in principle produced and controlled according to the current requirements (CPMP/ICH/95/95). The treatment with guanidine.HCl, which is performed before coupling the antibody to the resin, was investigated and showed some effectiveness for inactivation of the model retrovirus. There is no virus safety concern about the use of this antibody for the immune affinity chromatography.

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

The anticoagulant activity of activated protein C is easily demonstrated in *in vitro* clotting assays in which the addition of activated protein C [APC] to plasma produces a dose-dependent prolongation of clotting time. This is well demonstrated in the literature and probably reported best by Okajima et al. (1990), which showed a dose-dependent effect on the APTT of human plasma in the presence of a purified human protein C concentrate activated by snake venom as well as a purified thrombin-activated APC preparation. Other published reports on the anticoagulant activity of activated protein C include *in vitro* studies by Comp and Esmon (1979); and Marlar et al. (1982). *In vivo* experiments have also shown that infusion of species-specific APC in canine and bovine animal models prolongs clotting time in a dose-related manner as predicted from the *in vitro* experiments (Comp and Esmon, 1981; Comp et al., 1982).

It is difficult to evaluate the relevance of a species for preclinical testing of Protein C Concentrate on the basis of whether the test material is pharmacologically active due to the expression of the receptor because of the way in which the protein C anticoagulant pathway functions.

A further difficulty in evaluating the relevance of a species for preclinical testing of Protein C Concentrate is due to differences between humans and animals in phenotypical expression of severe protein C deficiency. A murine protein C knockout model (which in this case was not accessible) was first described by Jalbert (Jalbert et al., 1998). Mice with a total deficiency of protein C were produced from cross breeding between healthy protein C heterozygotes. Homozygous neonates with null protein C were morphologically normal, but died within 24 hours from severe thrombosis, demonstrating that the absence of protein C is not compatible with survival. This emphasizes the vital anticoagulant role of protein C in maintenance of homeostasis and survival. However in contrast to humans with severe protein C deficiency, they apparently had no signs of purpura fulminans in the skin. Thrombotic onset was primarily located in the brain at early developmental stages and progressed rapidly after birth. Neonates also demonstrated thrombotic manifestations in liver, kidney, heart and lung.

Due to these considerations, the preclinical safety-testing program was based on a variety of established animal models, which are used routinely by the company for testing other coagulation products. The choice of animal for each type of test was based on the specific sensitivities of the species in regard to the type of reaction being tested. For example, dogs and guinea pigs are particularly susceptible to anaphylactoid reactions. Dogs are also large enough to allow simultaneous testing of several parameters of vital function. The rabbit ear is particularly suitable for testing local tolerance because of the accessibility of long vessels, allowing macroscopic evaluation of changes. The Wessler rabbit stasis model has long been considered the classic model for testing the thrombogenic potential of test substances.

Pharmacodynamics

- General and safety pharmacology programme

Anaphylactoid potential

Guinea pigs

Male guinea pigs were anaesthetised and artificially respired. The animals were randomised to receive intra-arterial administration of one of the following test substances: one of two lots of Protein C Concentrate at a dose of 300 IU/kg, or an equivalent volume (3 ml/kg) of an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, Na_3citrate·$2H_2O$, and NaCl) or isotonic saline as a negative control. This design resulted in 4 experimental groups of 6 guinea pigs per group (total=24). A dose of 50 mg/kg immunoglobulin for intramuscular use was administered intra-arterially to each animal 20 minutes after the injection of the test substance as a positive control to ensure that an anaphylactoid reaction could be triggered in the animal.

Pulmonary inflation pressure was recorded continuously on a polygraph and evaluated before injection of the test or control article and 1, 3, 5, and 10 minutes after injection.

No positive reactions were seen after administration of Protein C Concentrate, albumin placebo, or saline control, although positive reactions were induced in all animals by the subsequent injection of the immunoglobulin positive control, demonstrating the validity of the model. Mean pulmonary inflation pressure essentially did not change 1 minute after administration of protein C, albumin placebo, or saline, but increased after administration of the positive control

A further study following a similar design investigated 3 lots of Protein C Concentrate. The results of this study did not show significant changes in respiratory pressure after intra-arterial injection of 300 IU/kg body weight of Protein C Concentrate, although subsequent injection of the positive control article led to significant increases in respiratory pressure.

The presented data investigating anaphylactoid properties do not give evidence of bronchospastic activity of protein C Concentrate in the animal model used.

Dogs

Spontaneously breathing male and female Beagle dogs were assigned randomly to receive intravenous administration of one of the following test substances: one of two lots of Protein C Concentrate at a dose of 500 IU/kg, or an equivalent volume (5ml/kg) of an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, Na_3citrate·$2H_2O$, and NaCl) or isotonic saline as a negative control. This design resulted in 4 experimental groups of 4 dogs per group (total n=16). A dose of 50 mg/kg immunoglobulin for intramuscular use was administered intravenously to each animal 180 minutes after the injection of the test substance as a positive control to ensure that an anaphylactoid reaction could be triggered in the animal.

The following variables were recorded before and 5, 15, 30, 60, 120, and 180 minutes after administration of the test or control article: mean arterial and pulmonary artery pressure, cardiac output, stroke volume, total peripheral vascular resistance, heart rate, respiration rate, respiratory minute volume, ECG, platelet count, and plasma fibrinogen concentration. The 180 min-value after administration of Protein C, albumin placebo, or saline served as the initial value for the evaluation of the immunoglobulin positive control.

The immunoglobulin positive control elicited changes in all variables except heart rate, serving to validate the responsiveness of the model. In contrast, no biologically relevant changes were seen with Protein C compared to albumin placebo and saline.

A further study investigated 2 lots of Protein C Concentrate using a similar design. In that study also there was no evidence of an adverse effect of Protein C Concentrate on any of the parameters tested.

The presented data investigating the safety pharmacological profile of the test substance do not give evidence of adverse effects of protein C Concentrate in the animal model used

Thrombogenic potential

Male and female New Zealand white rabbits were assigned randomly to groups of 8 animals per group: 6 animals in each group received an intravenous injection of 200 IU/kg of one of two lots of Protein C Concentrate or an equivalent volume of an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, $Na_3citrate \cdot 2H_2O$, and NaCl); 2 animals received an intravenous injection of an equivalent volume of activated prothrombin complex (20 U/kg) as a positive control (total n=24).

Thrombus formation was determined using the semiquantitative method described by *Wessler et al. (1959)*. The formation of thrombi was evaluated using a scoring system proportional to thrombus formation (0 = liquid blood without thrombi; 0.5 - 1 = a few small thrombi; 2 = several middle-sized thrombi or many small thrombi; 3 = a greater number of middle sized thrombi; 3.5 = few larger thrombi; 4 = large thrombus). Negative (non-thrombogenic) substances do not exceed a score of 1.

Protein C Concentrate (200 IU/kg i.v.) yielded a negative result (score 0 in all animals except one, which had a score of 0.5). The results for the albumin placebo were also negative, whereas scores for the positive control ranged from 3 to 4.

A further study, in which were tested 3 lots of Protein C Concentrate, all animals receiving Protein C had scores of 0.

It was concluded that the risk of thrombus formation after administration of Protein C Concentrate is negligible.

Immunogenicity studies

Studies on the immunogenic response were not conducted because prior experience with human plasma derived products had shown them to be of limited value. The difference between the recipient species and human protein C will inevitably result with antibody formation. Some information is available regarding immunogenic response of FVIII in mice. None of the normal mice developed detectable antibodies after the first injection, but 30 – 50% of the FVIII knockout mice did show antibody formation. After the second injection, anti-FVIII were detectable in all animals of both strains. There were no differences in final anti-FVIII antibody levels between FVIII knockout- and normal mice.

After intravenous treatment, the rate of antibody formation was very similar to that after subcutaneous treatment. This example of FVIII demonstrates that immunogenic studies in animals are of academic interest, but have no value in predicting an immune response against a therapeutic protein in humans. (Reipert et al., Thromb. Haemost., 2000.)

- Summary of salient findings

The usual dose for one injection of Protein C Concentrate in humans is between 40 and 100 IU per kg body weight. The maximum dose of Protein C Concentrate would not be expected to exceed 130 IU per kg body weight in a single injection. The maximum exposure in humans is expected to be approximately 100 IU/kg Protein C Concentrate given as a bolus 4 times daily over a maximum of 4 weeks during acute clinical situations, or approximately 1000 IU per day over longer periods after reaching a steady state.

The single doses used in the animal safety studies ranged from 100 to 6000 IU per kg body weight, i.e., far above the expected dose in humans. No indicatons of an anaphylactoid reaction were seen after intra-arterial administration of Protein C Concentrate at 300 IU/kg in guinea pigs. No biologically relevant changes were seen after intravenous administration of Protein C Concentrate at 500 IU/kg in dogs. No thrombus formation was seen after intravenous administration of Protein C Concentrate of 200 IU/kg in rabbits. No signs of toxicity were seen after intravenous administration at

2000 IU/kg in rats or 1500 IU/kg in mice. No morphological changes occurred at the injection site after intravenous, or intra-arterial injection into rabbits at a dose of 500 IU, or after paravenous injection of 100 IU.

Pharmacokinetics

The company states that pharmacokinetic testing of human proteins in experimental animals is not directly predictive of the situation in humans. Since the metabolic clearance rate depends on species-specific factors, Protein C Concentrate would be catabolized differently in heterologous than in homologous recipients. Thus, no animal studies were performed to address pharmacokinetics, distribution, biodegradation or excretion.

Toxicology

Single dose toxicity:
Rats
Male and female Sprague-Dawley rats were randomly assigned to groups receiving intravenous administration of one of the following test substances: one of two lots of Protein C Concentrate at a dose of 2000 IU/kg, or an equivalent volume (20 ml/kg) of an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, Na_3citrate·$2H_2O$, and NaCl) or isotonic saline as a negative control. This design resulted in 4 experimental groups of 10 rats per group (total n=40).

The animals were monitored for 14 days for unusual behaviour or physiological changes indicative of acute toxicity; body weight was measured on days 0, 7, and 14 to provide an indication of general health, and the number of deaths was recorded. On day 14 all surviving rats were sacrificed and a gross pathological examination was performed.

No deaths or signs of toxicity occurred in any of the groups treated with Protein C or saline. One male rat in the group receiving the placebo formulation showed behavioural depression and dyspnea for 5 minutes after administration. Based on this study, the No Observed Adverse Effect Level (NOAEL) for Protein C in rats is therefore at least 2000 IU/kg, which is approximately 20-fold the assumed maximum human dose for a single injection.

Similarly, no deaths or signs of toxicity were found in another study where 3 lots of Protein C Concentrate tested at doses of 1000 and 2000 IU/kg.

It was concluded that the safety margin between the human dose and the experimental doses is sufficiently large to qualify the human dose as safe. It is also stated thus the safety margin appears to be fully adequate in comparison with the dose of 100 IU/kg used in humans.

Mice
Male and female specific pathogen-free NMRI mice were randomly assigned to groups receiving intravenous injection of one of the following test substances: one of two lots of Protein C Concentrate at doses of 1500, 3000, or 6000 IU/kg, or equivalent volumes (15, 30, or 60 ml/kg) of an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, Na_3citrate·$2H_2O$, and NaCl) or 60 ml/kg isotonic saline as a negative control. This design resulted in 10 experimental groups of 10 mice per group (total n=100).

The animals were monitored for 14 days for unusual behaviour or physical changes indicative of acute toxicity; body weight was measured on days 0, 7, and 14 to provide an indication of general health, and the number of deaths was recorded. On day 14, all surviving mice were sacrificed and a gross pathological examination was performed. Organs showing abnormalities were preserved and subjected to histological examination.

A total of 13 deaths were observed in the group receiving 6000 IU/kg and 1 in the 3000 IU/kg group. Cramps, dyspnea and behavioural depression were observed after the administration of both protein C and albumin placebo, but not after saline. The symptoms in animals that died were indicative of citrate

toxicity, which was not surprising since the amount of citrate administered in 6000 IU/kg Protein C or 60 ml/kg albumin placebo exceeded the LD_{50} value of 260 mg/kg i.v. The No Observed Adverse Effect Levels (NOAEL) for Protein C Concentrate for this study in mice was concluded with 1500 IU/kg.

In another study with 3 lots of Protein C Concentrate, the NOAEL ranged from 1500 to 3000 IU/kg iv. The safety margin appears to be fully adequate in comparison with the dose of 100 IU/ml used in humans.

Repeated dose toxicity:
Repeated toxicity studies were not conducted because prior experience with coagulation preparations had shown them to be of limited value. Difference between the recipient species and human Protein C will inevitably result in an immune response with antibody formation.

Considering also the low level of impurities in the preparation and its biological identity to natural protein C, it was argued that repeat-dose toxicity studies at this stage in the development of the product would not furnish meaningful data which will impact on the way the product is used or labelled. Furthermore, data on long-term treatment in humans is available.

Genotoxicity:
Protein C Concentrate was tested for mutagenic activity with the "*Salmonella typhimurium* Reverse Mutation Assay" (Ames test).

All positive control groups showed significantly increased mutation frequencies, which demonstrates the sensitivity of the system. It was concluded that none of the tested concentrations of Protein C Concentrate showed a statistically significant increase of the mutation frequency compared to the control samples with any of the strains used. Metabolic activation did not change these results.

On the basis of the results obtained in this study it is concluded that the reconstituted solution of Protein C Concentrate is non-mutagenic in the Ames test with the strains *S typhimurium* TA97a, TA98, TA100, TA102 and TA1535 up to a concentration of 100 µl per plate.

Carcinogenicity:
Since humans treated with Protein C Concentrate receive native, homologous protein, carcinogenic effects are not to be anticipated. Repeated testing of human proteins in heterologous recipients is difficult because of the risk of formation of antibodies. Incompatibility reactions based on an antigen-antibody reaction would not be representative for the situation in humans. Therefore, in light of legislation restricting animal experimentation to meaningful and necessary tests, preclinical studies on carcinogenic potential were not conducted for Protein C Concentrate.

Reproduction Toxicity:
A teratogenicity study was initially not considered necessary because the main indication for Protein C Concentrate is in infants. However, since there may be clinical demand for the use of Protein C Concentrate in pregnant women with hereditary protein deficiency (the concentrate has been used in two pregnant women on a compassionate use basis), the SPC has been revised to clarify that very little information is available on the use of protein C concentrate in pregnancy.

Currently, experiments are conducted to investigate whether human protein C crosses the placental barrier in animals routinely used in reproduction toxicity studies: Five mice, 5 rats and 3 rabbits were intravenously administered Protein C Concentrate on the 12^{th}, 13^{th} and 15^{th} day of pregnancy, respectively. The dose was 2500 IU/kg in mice and rats and 1000 IU/kg in rabbits. 30 minutes after administration the anesthetized animals were sacrificed.

Protein C is found almost exclusively in the plasma of the parent animal and only in very low amounts in the organs examined. Moreover, it is uncertain whether the protein C found in the organs is to be attributed to the organs or to the blood they still contain. Protein C was never found in the embryos, irrespective of whether or not the parent animal was bled at the time of removal.

These studies suggest that the animal species studied are unsuitable for the determination of embryotoxicity and teratogenicity (performed mostly in rats and rabbits) of Protein C Concentrate.

Local Tolerance

Male and female New Zealand white rabbits assigned randomly to 9 groups of 4 animals (2 males and 2 females) per group, to receive either Protein C from 1 of 2 production lots or an albumin placebo control (equivalent to Protein C Concentrate in amounts of total protein, Na_3citrate·$2H_2O$, and NaCl). Injections were given either intravenously or intra-arterially into one ear at a dose of 500 IU, or paravenously at 100 IU. This design resulted in 9 experimental groups of 4 rabbits per group (total n= 36).

As a negative control, each animal received an injection of an equivalent volume of isotonic saline into the contra-lateral ear by the same route of administration. Following the injection, animals were monitored closely during the first 6 hours, then once a day during a total observation period of 72 hours for signs of local reactions, unusual behaviour, and any symptoms suggestive of acute toxicity. Thereafter, all animals were sacrificed. Injection sites were examined macro- and microscopically to assess the extent of any local tissue reactions.

No behavioural alterations were observed in any of the animals during the observation period. Virtually no pathological changes were found after administration of either Protein C Concentrate or albumin placebo. It was concluded that tolerance for Protein C Concentrate is excellent after intravenous, intra-arterial and paravenous administration in rabbits.

Similar results were obtained in another study with 3 lots of Protein C Concentrate.

The results obtained indicate that Protein C Concentrate is well tolerated in the animal model used.

Other toxicity studies

Safety of polysorbate 80

Studies of metabolism and excretion after intravenous application of polysorbate in experimental animals show that plasma lipases hydrolyze the polysorbate and the fatty acids undergo metabolism with CO_2 excreted via the lungs. The polyoxyethylene-sorbitan part of the molecule is excreted via the urine and partially via the bile in an unchanged manner *(CIR Expert Panel Report, 1984)*. There is good evidence that the metabolism of polysorbate is very similar in humans. McKean *et al.* (1987) showed identity by HPLC of the polyoxyethylene sorbitan metabolite in urine of humans after intravenous administration of polysorbate 20, and the analogous metabolite in urine of rats after intravenous treatment with polysorbate 80.

The *Cosmetic Ingredient Review Expert Panel* final report on the safety assessment of polysorbates *(CIR, 1984)* concluded that polysorbate 80 is nonmutagenic and noncarcinogenic (although polysorbates may enhance the activity of known chemical carcinogens), and that acute intravenous and intraperitoneal injection of polysorbates into rats and mice results in LD_{50} values indicative of only a low order of parenteral toxicity. Recent developmental toxicity studies showed no maternal or developmental effects of polysorbate 80 given intravenously at doses as high as 75 mg/kg from gestation day 0 to 19 in rats and 62.5 mg/kg from gestation day 7 to 19 in rabbits (Hilbish et al., 1997).

Reports in the literature vary as to polysorbate-induced human hypersensitivity. However, the most conservative data indicate that effects are seen only after administration of relatively large amounts of polysorbate 80. For example, small differences in hemodynamic parameters were observed after intravenous infusion of doses of 10 mg/kg polysorbate 80 administered in 5 mg/kg amiodarone, compared with the same amount of amiodarone without polysorbate 80 (Munoz et al., 1988). Sicart et al. (1977) observed hemodynamic changes in five patients after the administration of 20 mg/kg polysorbate 80 (analogous to the polysorbate content of 10 mg/kg amiodarone).

Since release criteria ensure that the polysorbate 80 content of Protein C Concentrate does not exceed 5 µg/ml, it was concluded that the safety margin is sufficient.

Impurities/Metabolites:

Evaluation of possible formation of neoantigens after vapour heating of Protein C
To reduce risk of virus transmission, Protein C is subjected to vapour heating. The increased temperature used during this treatment may theoretically result in the formation of novel proteins. A study was therefore carried out to evaluate the influence of vapour heating on the molecular integrity of Protein C Concentrate by determining whether neoantigens are present after vapour heating of the product (e.g., 10 h at 60°C plus 1 h at 80°C).

Rabbits were immunized with three batches each of untreated or vapour heated intermediate bulk powder. The resulting antiserum after immunization with the vapour heated product were used both in native form and absorbed with the untreated product. Similarly the resulting antisera after immunization with the untreated product were used native and absorbed with the vapour-heated product, to demonstrate disappearance of antigens.

The test and control articles were electrophoretically separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were electrotransferred (Western Blot) to a nitrocellulose (NC) matrix, and incubated with the respective rabbit antisera against the vapour heated product, or against the untreated product with and without absorption, to form immune-complexes on the matrix. After incubation with an affinity-purified goat anti-rabbit IgG, coupled to horseradish peroxidase, the immuno-complexes were stained by incubation with 4-chloro-1-naphtol and hydrogen peroxide and evaluated visually.

Neither changes -neither formation of neoantigens nor disappearance of antigens originally present in the untreated product- were detectable after vapour heating of Protein C Concentrate.

CEPROTIN also contains trace amounts of mouse protein. The concentration of murine IgG of ≤ 3ng/ml is smaller than that usually contained in other therapeutic products purified by monoclonal antibody affinity chromatography, such as highly purified FVIII concentrates or recombinant FVIII concentrates, which are safely administered on a life-long basis.

The benzamidine concentration in CEPTROTIN is ≤ 40 ng/100 U. Analysing DNA single strand breaks in rat hepatocytes in vitro, benzamidine was without effect at concentrations corresponding to 290 mg/kg *in vivo*. Between the concentration used *in vitro*, which remained without gene toxic effects, and the benzamidine exposure of 52 ng/kg of a patient who receives CEPROTIN there is a difference of 1:5 577 000. Therefore, the safety margin is sufficient. In addition, it should be noted that the Ames test performed with CEPROTIN was negative.

CEPROTIN contains ≤ 20 ng/ml EDTA corresponding to a single human maximum dose of ≤ 26 ng/kg. EDTA as a stand alone therapeutic is intravenously infused as an antidote in patients in doses up to 80 mg/kg per day up to 5 days, repeated if necessary after an interval of 2 days. The maximum dose given is 0.8 g/kg (Martindale, 1993). Therefore, the trace amounts of EDTA contained in CEPROTIN are several orders of magnitude lower than that usually given intravenously to a patient treated specifically with EDTA. The EDTA concentrations in CEPROTIN can be considered as of no toxicological relevance.

Discussion on toxico-pharmacological aspects

A series of preclinical safety studies were carried out with Protein C Concentrate manufactured according to the modified process (Method II). Studies investigating special pharmacodynamic and pharmacokinetic aspects were not applied. Reprotoxicity, in vivo-mutagenicity and cancerogenicity studies were not performed due to the pharmacological profile of the product and the human origin of the active substance.

All pivotal safety studies with regard to anaphylactoid reactions in guinea pigs and dogs and coagulation activation in dogs, thrombogenic potential in rabbits, acute toxicity in rats and mice, and local tolerance in rabbits, were not shown to raise any safety concerns. Investigation of local tolerance in rabbits indicated that the product was well tolerated after intravenous, intra-arterial, and paravenous injection. In vitro studies on mutagenicity and molecular integrity did not reveal safety concerns.

No studies were performed to address pharmacokinetic properties. However, the company states that such information does not appear necessary for Protein C Concentrate because of the nature of the product as a homologous replacement protein, particularly in view of the clinical data already available.

The lack of repeated dose toxicity, carcinogenicity and reproductive studies can be considered acceptable in view of the fact that Protein C is an endogenous protein which would induce the development of neutralising antibodies in heterologous recipients.

Review of the literature on the safety of polysorbate 80 indicates that the use of this substance in the production of Protein C Concentrate poses no safety concerns, since a relatively large safety margin exists at the proposed specified residue limits.

The responses submitted by the company to the questions concerning the pharmacological and toxicological documentation were regarded to be sufficient and the objections were clarified.

Considering the results of the preclinical documentation it can be concluded that there is no evidence for an adverse pharmacological and toxicological potential of CEPROTIN regarding safety concerns for the administration to humans.

4. Clinical aspects

Three clinical study reports have been submitted to support the licensure of Protein C Concentrate:

- An interim report on a clinical study (IMAG-098) entitled "*A Clinical Study on the Pharmacokinetics of PROTEIN C CONCENTRATE in Asymptomatic Subjects with Homozygous or Double Heterozygous Congenital PC Deficiency*". This summarises pharmacokinetic data and acute safety data from eight asymptomatic subjects. Method II produced PROTEIN C CONCENTRATE for this clinical study, which is being performed in full compliance with current GCP guidelines.

- A compilation of data retrospectively collected from
  a) Compassionate treatments of subjects with congenital or acquired PC deficiency;

  b) A clinical study (IMAG-039) entitled "*An Open Label Study of the Efficacy and Safety of PROTEIN C CONCENTRATE in the Treatment of Severe Congenital Protein C Deficiency with or without Purpura Fulminans*". This study included 79 patients. Twenty-two subjects with homozygous or double heterozygous PC deficiency, with or without thrombotic events such as purpura fulminans, with arterial and/or venous thrombotic disease, were evaluated for efficacy;

  c) Two subjects with homozygous PC deficiency from a clinical study (IMAG-041) entitled "*An Open Label Study on the Efficacy and Safety of PROTEIN C CONCENTRATE in the Treatment of Warfarin (Coumarin)-Induced Skin Necrosis with PC Deficiency*", were evaluated for safety.

Since treatments cover a period from 1990 to the present, the product used was produced by Methods I and II. Study treatments and compassionate uses do not always conform to the current standards for GCP, since treatment was provided in life-threatening situations. Data sets for individuals are incomplete because of the retrospective character of the evaluation.

- A Phase I/II study conducted from 1989 to 1992 summarises the initial experience with PROTEIN C CONCENTRATE (produced by Method I) in humans. The report includes treatment information for eight patients; six with congenital PC deficiency, one with acquired deficiency and one with deficiency of unknown origin. Data were analysed for acute safety and tolerability, and initial pharmacokinetic data were obtained. In four subjects with acute thrombotic complications, preliminary evidence of efficacy in terms of improvement of thrombosis/skin necrosis was described. The study was conducted in accordance with Good Clinical Practice guidelines and national requirements in effect in Germany at the time.

Clinical pharmacology

Protein C is the zymogen of a coagulation inhibitor. Congenital deficiency of protein C is an autosomal disorder and associated with increased risk of venous thrombosis. The majority of the patients are heterozygous for the defect. Heterozygous protein C deficiency is characterised by an increased risk for thromboembolic disease as encountered in heterozygous antithrombin III deficiency.

Homozygous protein C deficiency is rare: only 17 cases of homozygous type 1 protein C deficiencies are recorded in an international database of mutations and only about 20 further cases of homozygous protein C are reported in the literature. Most commonly these patients present with purpura fulminans in the neonatal period. Typically, these infants have protein C levels that are less than 0.10 units/ ml (U/ml). A small number of severe protein C deficient patients do not present with purpura fulminans at birth. Rather they present with severe thromboembolic disease during childhood or early adult life. To date only 8 patients presenting with late onset of thromboembolic complications have been confirmed by DNA analysis to be true homozygotes.

Double heterozygous defects, a separate defect on each pair of protein C genes, can also result in severe protein C deficiency with symptoms similar to those in homozygous protein deficiency.

Pharmacodynamics
Mechanism of action

Protein C is a vitamin K-dependent anticoagulant glycoprotein that is synthesised in the liver. It is converted by thrombin/ thrombomodulin-complex on the endothelial surface to activated protein C (APC). APC is a serine protease with potent anticoagulant effects, especially in the presence of its cofactor protein S. APC exerts its effect by the inactivation of the activated forms of factors V and VIII, which leads to a decrease in thrombin formation. APC has also been shown to have profibrinolytic effects.

Pharmacokinetics
General

IMAG-098: Clinical study on the pharmacokinetics of Protein C Concentrate in asymptomatic subjects with homozygous or double heterozygous congenital protein C deficiency

The objective of the study was to determine the pharmacokinetic (pk) parameters of Protein C Concentrate in asymptomatic patients with homozygous or double heterozygous protein C deficiency. In addition, acute safety of the product was assessed. An interim report on the clinical study was submitted and evaluated.

Eight to 12 asymptomatic patients with homozygous or double heterozygous protein C deficiency, who may be receiving protein C replacement and/ or anticoagulants at the time of entry, were planned to be recruited into the study. To date 13 subjects are enrolled.
Patients meeting the following inclusion/ exclusion criteria were included in the study:

Inclusion criteria:

- have provided written informed consent (if subject is ≤18 years of age, parent or legal guardian must sign and date an informed consent form);
- have a diagnosis of congenital protein C deficiency (homozygous or double heterozygous);
- are asymptomatic;
- are allowed to receive oral anticoagulants;
- if female of child bearing potential, a negative pregnancy test must be documented within 1 week before administration of the test dose.

Exclusion criteria:

- body weight less than 8 kg;
- active liver disease defined as an ALT level greater than 2.5 times the upper limit of normal for the testing laboratory;
- any thrombotic complications within two weeks prior to administration of the test dose;
- receiving an investigational product other than Protein C Concentrate within 60 days prior to the infusion of the test dose.

Subjects on prophylactic treatment with Protein C Concentrate at the time of study entry receive their last prophylactic infusion 30 to 36 hours prior to the administration of the test dose for pk assessment. Protein C levels are to be determined in all subjects weighing more than 15 kg, pre-infusion and at 1, 17 and 25 hours post-infusion.

All subjects receive Protein C Concentrate as a single test dose of 80IU/ kg for determination of the pk parameters.
Samples for determination of protein C are drawn pre-infusion and at 30 minutes, and 1, 2, 4, 8, 12, 24, and 36 hours. Subjects with severe protein C deficiency who are currently on a regimen involving treatment every 24 or 48 hours do not have the 36 hour sample drawn..
At the administration of the test dose and the pre-test dose respectively the subject's vital signs are recorded pre-infusion, at 30 minutes, 2, 4 and 24 hours post-infusion. Blood samples are drawn for local laboratory assessments (whole blood count), pre-infusion, at 30 minutes and 24 hours post-infusion.

The functional protein C activity is measured using a chromogenic assay. At baseline and at 3 month, blood is drawn to test for viral markers and antibody development. Additionally, blood is drawn for Polymerase Chain Reaction (PCR) testing (HIV1/2, HBV, HCV and PVB-19). The detailed test descriptions and the validation of the principal of the test methods for protein C activity, inhibitory antibodies against protein C, anti-murine antibodies, viral markers and blood count and haematocrit have been provided.
Twelve asymptomatic subjects with homozygous or double heterozygous protein C deficiency were evaluated for pk data.

Two models were used in the analysis of the data: the compartmental approach described by Lee, Poon and Kingdon and the non-compartmental approach described by, among others, Morfini and colleagues. There is no clear answer whether the model used fits the data best. For the non-compartmental approach there is no fit to the observed concentrations whereas for the compartmental model used to derive the terminal half-life is considered to be rather vague. The compartmental model used for each individual patient is specified; the choice for each patient (i.e whether 1 or 2 compartmental) is described.

Compartmental and non-compartmental $t_{1/2}$ were calculated in the same patients and thus methods for paired data had to be applied. In each subject, the half-life computed by the non-compartmental method is longer than that computed by the compartmental approach (median: 5.25 hours). A Wilcoxon Signed Rank test indicates that the difference between both methods is statistically significant ($p = 0.0005$).

Twelve asymptomatic subjects with homozygous or double heterozygous protein C deficiency were evaluated for pk data. Some of the subjects with severe protein C deficiency are currently on a

regimen involving treatment every 24 or 48 hours. The sponsor's decision to use a wash-out period of only approx. 3 half-lives combined with a limited amount of Protein C Concentrate for the last prophylactic treatment is matter for concern. In several cases the post observation period was shortened to 24 hours (less than 3 half-lives). A foreshortened post-infusion observation period could reduce the half-life estimates.

Two subjects were identified with APC resistance in study IMAG-098, and their in-vivo half-lives of Protein C were found to be in the range described for the overall study group. The results reveal that the pharmacokinetics of Protein C have such a high inter-subject variability that APC resistance is most likely not to be considered as a confounding variable.

The clinical study on the pharmacokinetics of Protein C Concentrate in asymptomatic subjects with homozygous or double heterozygous congenital protein C deficiency revealed a marked variability of the pk parameters between the individual patients, eg. Half-life: median 10.6 hours, range 4.4-15.9 hours, and incremental in vivo recovery: median 0.014 IU/ml per IU/kg, 0.005-0.17 IU/ml per IU/kg, The pharmakokinetic data are not sufficient to characterise the product and to give recommendations on dosage and frequency of administration of Protein C Concentrate.

There is no discussion on the clinical impact of the differences.

The data on the history of thrombotic events for the patients who are currently on prophylaxis were not provided with the original submission, but have been requested to be included in the final study report.

Seven of the 13 study subjects were not on prophylactic treatment with Protein C Concentrate. All of these 7 subjects received oral anticoagulant therapy for prophylaxis. Three of the 7 subjects received Protein C Concentrate on demand for thrombotic episodes prior to enrollment and 4 subjects did not receive Protein C Concentrate at any time during the last two years prior to enrollment. Two of these 7 subjects had their last thrombotic episode more than 2 years prior to enrollment into the study: one subject had a DVT, for this subject the treatment for this event is not known, and one subject had microthrombosis on the skin and received Protein C Concentrate on demand. The other 5 subjects had thrombotic episodes during the last 2 years prior to enrollment. Two subjects received Protein C Concentrate for treatment of acute episodes, and three subjects received Fresh Frozen Plasma.

The data on the history of thrombotic episodes and treatment for the 7 subjects that were not on prophylactic treatment with Protein C Concentrate, during the two years prior to study entry are provided. The data on the history of thrombotic events for 6 patients on prophylaxis are also of importance, and these data should be included in the final study report.

Additional pharmacokinetic data:
A study report "Clinical Study of Phases I and II for assessment of efficacy, tolerability, and in vivo-recovery of Protein C Concentrate in patients with congenital protein C deficiency", presents an analysis of pharmacokinetic parameters in 5 patients with congenital protein C deficiency. Protein C Concentrate manufactured by method I was used for this study.

The median half-life in this study was 12 hrs (two phase model) and 14 hrs (non-compartmental). These results were in accordance with previous findings published in literature. However it has to be taken into consideration that the pk data published in literature were obtained in few patients all under therapy with Protein C Concentrate manufactured by Baxter AG, so to make a comparison between these data is not very meaningful.

Additionally study IMAG-39 includes analyses of pharmakokinetic parameters for 6 patients. These pharmakokinetic data are not comparable with the pk data obtained in study IMAG-098, since the protocol for pk evaluation was not standardised due to the clinical situation.

Clinical efficacy

Dose response study

No formal dose-finding studies were performed.

Report on a Retrospective Data Collection Conducted for Clinical Study IMAG-039, Treatments Under Compassionate Use Provisions and Two Subjects Treated Under Protocol IMAG-041

This retrospective data collection concerned the centres that in the past had used PROTEIN C CONCENTRATE in the United States, Canada and Europe, either under compassionate use, in study IMAG-039 or in study IMAG-041.

Data from two subjects treated under study IMAG-041 were collected and are reported in the Safety section of this report

1. Description of the study

According to the protocol, study IMAG-039 was an open label study to assess Protein C Concentrate as an effective and safe replacement therapy for severe congenital protein C deficiency in children and to make it available to patients worldwide who is without other adequate treatment alternatives. Study participation was open to subjects:

- with arterial and/or venous thrombotic disease;
- with a diagnosis of homozygous or double heterozygous protein C deficiency;
- with or without purpura fulminans;
- of any age or sex;
- providing informed consent.

The only exclusion criteria were in the case that subjects or parents or legal guardians refuse to give informed consent.

Laboratory tests included a complete blood count, several parameters to assess the coagulation status and viral safety markers.

Data on 79 subjects with congenital or acquired PC deficiency were collected. A subset of 21 subjects with a diagnosis of homozygous (n=16) or double heterozygous (n=5) PC deficiency was evaluated for efficacy. Eighteen were treated intravenously with PROTEIN C CONCENTRATE. Three treated under compassionate use had initially received the product intravenously but were switched to subcutaneous treatment later on.

According to protocol, subjects were to receive a test dose of 10 U/kg b.w., infused over a 20-minute period, during which the subject was monitored for adverse events including allergic reactions to the product. If no adverse or allergic reactions were noted, the subjects were to be given an initial therapeutic dose of 40 IU/kg b.w, infused over 15-20 minutes. The therapeutic dosage for further infusions had to be adjusted on the basis of half-life studies performed with the goal of achieving a twice-daily or once-daily treatment. The planned level of PC in these subjects was above 0.8 IU/ml (80% of normal). Treatment with PROTEIN C CONCENTRATE was to be continued at least until the disappearance of skin lesions and until oral coagulation could be safely started. The treatment period varied from 1 to 2,241 days.

For the initial therapeutic dose, blood samples were drawn to establish the appropriate level for subsequent doses of PC for each individual. Serial samples were taken pre-infusion and 15 minutes, 30 minutes, 1 hour, 4 hours, 8 hours, and 12 hours post-infusion.

Monitoring during the acute phase comprised laboratory tests and a physical examination on a daily basis to check. During steady state, the physical examination was carried out every second day and PC

activity levels were determined every third day. Antibodies to PC and murine immunoglobulin were reportedly determined one month after starting treatment and then at six months.

1. Primary endpoints/assays

The planned endpoints of the study were normalisation of protein C levels and laboratory parameters indicating coagulation activation, the dissolution of thrombotic occlusions, and the regression of any skin lesions present at entry.

The evaluation included treatments for acute symptoms, 12 courses for short-term prophylaxis prior to surgery or invasive therapy, and five courses of long-term prophylaxis of thrombotic events or complications.

2. Study populations/accountability of patients

In the study protocol the parameters to be included in the patient entry form were specified. This parameters include: diagnosis of congenital protein C deficiency, protein C levels at time of diagnosis, medication, including vitamin K prophylaxis at birth, history of venous or arterial thrombotic disease, and purpura fulminans, history of pregnancy. If possible, a family study to confirm the presence of inherited protein C deficiency had to be conducted. Furthermore a physical examination and laboratory test had to be performed at the time of study entry. Existing skin lesions had to be documented by photographs. Ultrasonogram of the abdomen was recommended, as well as an evaluation of the presence of renal vein thrombosis and. When feasible, CT scans of the head and abdomen was advised.

3. Efficacy results

The data of seventy-nine patients were analysed for the retrospective data collection, the outcome of 60 patients have been presented. Twenty-two of these patients were diagnosed with homozygous or double heterozygous protein C deficiency, 5 patients with heterozygous protein C deficiency, 10 patients with acquired protein C deficiency and one patient with multiple congenital deficiencies. For 20 patients no diagnosis was specified. Twenty-two subjects with either homozygous or double heterozygous protein C deficiency were evaluated for efficacy.
Efficacy data were presented on basis of the investigators overall assessment. No efficacy assessment based on physical examination or laboratory tests was provided.

In patients with severe congenital PC deficiency protein C concentrate improved all 16 purpura fulminans and all six coumarin-induced skin necrosis episodes, but only three of the other 15 thrombotic events. In patients with single heterozygous, acquired or unknown deficiency, treatment with Protein c Concentrate improved only less than half the latter episodes. The treatment outcome for the treatment of purpura fulminans and episodes of coumarin-induced skin necrosis suggests a benefit of the treatment with Protein C in patients with severe congenital protein C deficiency or in patients with single heterozygous, acquired and unspecified protein C deficiency. However the data are insufficient to suggest that the outcome of other thromboembolic events then purpura fulminans and episodes of coumarin-induced skin necrosis can be influenced by treatment with protein C.

However due to the low patient numbers and the nature of the study the effect of protein C treatment cannot be assessed finally.

Six subjects received 12 short-term prophylactic treatments prior to surgery, for other invasive procedures or under conditions of increased risk of thrombosis (e.g. pregnancy/childbirth). Baseline protein C level was available for 6 patients, the values ranged from 3% to 34%. However it has to be noted that different methods of protein C determination were used. No information about target level for protein C is available. All patients had thrombotic events in medical history. During short-term prohylaxis and anticoagulation with either heparin or coumarine no thrombotic episode during treatment was observed. An extreme variability in dosage and frequency for protein C administration

can be noted. There was one thrombotic complication reported in a patient who developed an asymptomatic catheter thrombosis in association with an infected central line.

Supportive study

Clinical Study of Phases I and II for assessment of efficacy, tolerability, and in vivo-recovery of Protein C Concentrate in patients with congenital protein C deficiency

The open label phase I/ II study was conducted from 1989 to 1992 to obtain initial clinical experience with Protein C Concentrate produced by method I with regard to the safety and tolerability.
In phase I of the study, the primary objective was to assess acute safety and tolerability of Protein C Concentrate in subjects with congenital protein C deficiency. Efficacy was also assessed in symptomatic subjects. A secondary objective was the determination of in-vivo recovery and half-life of Protein C Concentrate.

In phase II, efficacy in terms of improved skin necrosis and/ or thrombosis was evaluated. Safety and tolerability were secondary objectives.

The following inclusion criteria were used in the selection of patients:

- diagnosis of congenital protein C deficiency with skin necrosis (purpura fulminans, coumarin-induced necrosis) and/or thrombosis;
- diagnosis of congenital protein C deficiency and who were in the initial phase of oral anti-coagulation therapy, consequently having an increased risk of thrombosis and/or skin necrosis;
- informed consent obtained from subject or legal guardian following information on nature, significance and consequences of the clinical study by a physician.

The following exclusion criteria were used in the selection of patients:

- pregnancy;
- no informed consent obtained from subject or legal guardian;
- contraindications for treatment with Protein C Concentrate
- known allergic reactions to human blood products or murine immunoglobulins.

There were no pre-determined reasons for removal of individual subjects from therapy or assessment.

It was predetermined that the study would be terminated if severe anaphylactoid or anaphylactic reactions occurred during or after infusion of Protein C Concentrate or if other severe adverse experiences occurred that were possibly caused by the administration of Protein C Concentrate.

Phase I was designed as a single-arm, increasing-dose, safety study in up to 10 subjects with congenital protein C deficiency. Three infusions were given: the first infusion of 5 U/kg bodyweight, a second infusion of 20 U/kg bodyweight 120 minutes later and a third infusion of $\leq$ 40 U/kg bodyweight three or more days later. Following the infusions, subjects were observed for anaphylactoid/ anaphylactic and hypotensive reactions for a period of 120 minutes. Monitoring for adverse experiences was performed throughout the study period. Blood samples for pharmacokinetic analysis were drawn before the last infusion and 15, 30 and 60 minutes, 2, 4, 8, 24, 36, and 48 hours thereafter. Analytical methods to be used were not specified in the protocol. Protein C antigen and activity were determined using commercial available test systems and according to procedures established in the local laboratories.

Phase II was designed as a single-arm efficacy study at varying doses to achieve a protein C level at 70% of normal. Clinical efficacy in terms of improvement of skin necrosis/thrombosis, safety, and tolerability was to be assessed in symptomatic patients. The dosage was calculated on the basis of the half-life and in-vivo recovery determined in phase I.

Patients previously in phase I who developed skin necrosis and/or thrombosis was able to receive treatment with Protein C Concentrate within one year after the onset of the study. The treatment was administered as soon as possible at doses that would result in protein C plasma levels of 70% of normal. It was assumed that the administration of 1 IU of protein C per kg bodyweight raises the

protein C activity by approximately 1.3%. Depending on the calculated half-life, further administrations of Protein C Concentrate were given until the symptoms disappeared. Phase II also included testing for viral safety over a period of 6 months.

Primary endpoints/assays
The clinical endpoint was defined as clinical efficacy (descriptive; in terms of improvement of skin necrosis/thrombosis) at a level of protein C activity of 70% of normal.

Efficacy results
Six patients out of nine enrolled presented acute symptoms and were evaluated for efficacy: 2 with thrombosis, 1 with thrombosis and skin necrosis, 1 with necrosis at the galea aponeurotica, 1 with haematoma, and 1 with haemarthrosis. In all cases the symptoms improved or disappeared with PROTEIN C CONCENTRATE. A one-year-old male with acquired PC deficiency of unknown origin was also included in the trial report. He had a history of myocardial infarction and vena cava thrombosis. At entry he presented necrosis of the galea aponeurotica. He received a single infusion of PROTEIN C CONCENTRATE (65 U/kg), which almost completely healed the necrosis on the scalp.

Discussion on clinical efficacy

The clinical study on the pharmacokinetics of Protein Concentrate in asymptotic subjects with homozygous or double heterozygous congenital protein C deficiency revealed a marked variability of the pk parameters between the individual patients. The pharmakokinetic data are not sufficient to characterise the product and to give a recommendations on dosage and frequency of administration of Protein C Concentrate.

Study IMAG-098
The study IMAG-098 continues either until subjects can be transferred to a new study or licensure is obtained. Six of the 13 subjects were on continuous prophylactic treatment with Protein C Concentrate. Three subjects experienced thrombotic events while on prophylactic treatment with Protein C Concentrate. The events occurred while changing the treatment schedule - in one case after an attempt to stop oral anticoagulation. In the two other cases the treatment schedule for Protein C Concentrate needed to be changed.

Study IMAG-039, IMAG-041 or treatment with Protein C for compassionate use
- Treatment for acute thrombotic events in subjects with severe congenital protein C deficiency and treatment for acute thrombotic events in subjects with simple heterozygous, acquired and unspecified protein C deficiency:

In patients with severe congenital PC deficiency protein C concentrate improved all 16 purpura fulminans and all six coumarin-induced skin necrosis episodes, but only three of the other 15 thrombotic events. The treatment improved less than half the latter episodes also in patients with single heterozygous, acquired or unknown deficiency. However due to the low patient numbers and the nature of the study the effect of protein C treatment on thrombotic events other than purpura fulminans or coumarin induced skin necrosis can not be assessed finally.

Long-term prophylaxis
Nine patients are included in the subset of patients on long- term -prophylaxis. Eight of the patients are under anticoagulation with coumarin and prophylaxis with Protein C. For 4 patients thrombotic episodes are reported during long- term treatment. In three patients, these episodes are reportedly due to interruption of Protein C treatment, in one case, mild thrombotic lesions occurred probably as a consequence of an injury.

Short-term prophylaxis
Six subjects received 12 short-term prophylactic treatments prior to surgery, for other invasive procedures or under conditions of increased risk of thrombosis (e.g. pregnancy/childbirth). All patients had thrombotic events in their medical history. During short-term prohylaxis and anticoagulation with either heparin or coumarine no thrombotic episode during treatment was observed. An extreme

variability in dosage and frequency for protein C administration was noted. There was one thrombotic complication reported in a patient who developed an asymptomatic catheter thrombosis in association with an infected central line.

Clinical safety

A comprehensive summary report has been provided of adverse events, (including deaths, arrhythmia, chest pain, vein thrombosis), ECG and laboratory data (including anti protein C antibody, viral safety data and liver function) in all treated patients independently from their diagnosis and the study in which they were involved.

Adverse Experiences

Allergic reactions:
Ten cases of allergic symptoms including hot flushes (1) rash (4) hives (2), erythema, (1), itching (1), papular rash(1) were reported.

Bleeding:
Twelve cases of adverse events indication a bleeding episode were reported. These reports include bruising (3), gastrointestinal tract bleeding (1), haematemesis (1), hypocoagulable state (1), nosebleed (1), purpura (1), pulmonary haemorrhage (1), blood in urine (1), cerebral haemorrhage (1), subcuteanous haematoma (1).

Thrombosis:
Nine cases of thrombotic events were reported (thrombosis (7), peripheral ischemia (1) and thrombosis vena cava inferior (1)).

Deaths:
Eight deaths occurred in the study IMAG-039, IMAG-041 or under compassionate use. Short narratives of this case are provided. None of the death were rated to be related to the study drug.

One episode of arrythmia, 4 episodes of tachycardia, 3 episodes of hypotension (one of them severe), 1 episode of hypertension, 2 episodes of chest pain and 8 episodes of thrombosis, including vein thrombosis) were reported; none of the symptoms were considered related to the study drug.
The most common adverse experience, reported 9 times in a single subject (RDC; subject 0006) with severe congenital protein C deficiency, was sepsis from a central venous catheter.

Viral Safety
The clinical assessment of the viral safety of protein C concentrate is mainly derived from data evaluated in the framework of the Retrospective Data Collection. Data on HBs-Ag, HIV antibodies and hepatitis C antibodies were available for 53 of 79 study subjects. Baseline samples were available for 29 subjects. For 25 of 53 subjects, the viral follow-up covered 6 months to 7 years of Protein C Concentrate treatment. One subject (0069) was found to be HBsAg positive in 1997. Prior to 1997, the subject received a large amount of Fresh Frozen Plasma (FFP) and some infusions of CEPROTIN. Since there were no baseline measurements for hepatitis B antigen or antibodies for this subject, there was insufficient data to conclude that the seroconversion was not related to the administration of CEPROTIN. However, no other patient who received that lot or any other lot of CEPROTIN seroconverted for hepatitis B.

In the pharmacokinetic study, screening for antibodies and viral genome (by Polymerase Chain Reaction, PCR) for the viral marker human immunodeficiency virus types 1 and 2, hepatitis A, B and C and parvovirus B-19 were performed in 13 subjects. No related viral transmission or seroconversion was observed in any subjects.

Inhibitory antibodies against Protein C and Anti-murine Antibodies
Fifteen subjects were evaluable for the assessment of development of inhibitory antibodies against protein C. The subjects received between 2 to 2,386 Protein C Concentrate infusions, with the total

amount of product received per subject ranging from 15,840 to 2,160,600 IU Protein C Concentrate. No subject was shown to have developed inhibitory antibodies against protein C.

Seven subjects were followed for anti-murine antibodies between 17 days and approximately 4 years after the first Protein C Concentrate administration. No baseline samples were available. All except one subject tested negative for anti-murine antibodies. This subject had a test result of 30ng/ml approximately 2 months after the first product administration. This test result is within the normal range, as determined in a cohort of healthy individuals.

Discussion on clinical safety

A summary of all adverse signs/symptoms in all three clinical studies has been provided.
One hundred forty five adverse signs/symptoms (including eight deaths, none of which were considered related to the study drug) were reported in all clinical studies (Phase I/II, Retrospective Data Collection and Pharmacokinetic Study, IMAG-098).
However, information of the onset date of the AEs in relation to the infusion date is not provided. Therefore, a final assessment of the relation of the AE to the study drug was not possible. However due to the nature of the product for the following AEs a at least theoretical relationship to the study drug should be considered: Allergic reactions (10 cases), bleeding (12 cases) and thrombosis (9 cases).

5. Overall conclusion and benefit/risk assessment

- **Quality**

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. The manufacture of the product is performed using appropriately validated processes, which consistently yields a product of quality.

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

Overall, the primary pharmacological and toxicological documentation studies provided were considered to be adequate.

- **Clinical efficacy and safety**

CEPROTIN suggests a benefit for the treatment of purpura fulminans and coumarin induced skin necrosis in patients with severe protein C deficiency.

Twelve courses of short-term prophylaxis prior to surgery or invasive therapy and 9 courses of long-term prophylaxis were included in the efficacy analyses.

Nine patients are included in the subset of patients on long- term -prophylaxis. Of them 8 are under anticoagulation with coumarin and prophylaxis with Protein C. For 4 patients thrombotic episodes are reported during long- term treatment. In three patients, these episodes are reportedly due to the interruption of Protein C treatment, in one case, mild thrombotic lesions occurred probably as a consequence of an injury

Safety

A summary of all adverse signs/symptoms in all three clinical studies was submitted.
In the pharmacokinetic study, screening for antibodies and viral genome by Polymerase Chain Reaction, (PCR) for the viral marker human immunodeficiency virus types 1 and 2, hepatitis A, B and C and parvovirus B-19 were performed in 13 subjects. No related viral transmission or seroconversion was observed in any subjects.

Twenty-two subjects were evaluated for the assessment of development of inhibitory antibodies against protein C. All except one subject tested negative for anti-murine antibodies. This subject had a test result of 30ng/ml approximately 2 months after the first product administration. This test result is within the normal range, as determined in a cohort of healthy individuals.

The safety profile is acceptable.

Benefit/risk assessment

Since the efficacy/safety profile of this product has not been fully established yet, its use is deemed relatively safe and effective only in the severe clinical conditions for which it is indicated.

The submitted data confirm that the marketing authorization can be granted under exceptional circumstances.

1. Baxter commits to perform a prospective clinical study in patients with severe congenital protein C deficiency. This will be a combined phase I/II study to evaluate:

- Pharmacokinetic properties of CEPROTIN
- Evaluation of safety and efficacy.
- In the treatment of acute episodes of purpura fulminans and/or coumarin induced skin necrosis.
- In the prevention of acute episodes of purpura fulminans and or thrombotic complications in situations of high risk.

This includes dose finding with assessment of markers of coagulation activation such as D-dimers and thrombin-antithrombin complex. A separate dosing range (3 doses) will be determined for asymptomatic and symptomatic patients.

The study is estimated to be initiated by Q4 2001 and will take approximately 2 years for completion. Baxter commits to provide annual interim reports.

Baxter will apply for scientific advice before the end of May 2001.

2. The company commits to monitor and report to CPMP all treatment courses as completely as possible. Special emphasis will be put on prophylactic use in homozygous and double heterozygous patients, especially newborns. The company commits to include the sales figures in the PSUR.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered favourable by consensus that the benefit/risk profile of CEPROTIN in the treatment of purpura fulminans and coumarin induced skin necrosis in patients with severe congenital protein C deficiency. Furthermore CEPROTIN is indicated for short-term prophylaxis in patients with severe congenital protein C deficiency if one or more of the following conditions are met:

- surgery or invasive therapy is imminent
- while initiating coumarin therapy
- when coumarin therapy alone is not sufficient
- when coumarin therapy is not feasible.