SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Xigris. This scientific discussion has been updated until 1 October 2004. For information on changes after 1 October 2004 please refer to module 8B.

1. Introduction

Sepsis is an infection-induced syndrome defined as the presence of two or more features of systemic inflammation (such as fever or hypothermia, leukocytosis or leukopenia, tachycardia, tachypnoea or supranormal minute ventilation). Severe sepsis is defined as being sepsis associated with acute organ dysfunction and it results from a generalised inflammatory and procoagulant response to an infection. Current treatment of sepsis is largely based on administration of antibiotics, source control and supportive treatment (fluids, inotropes, vasopressors, replacement of failing organs). The death rates in some subgroups of patients with sepsis-induced organ failure have decreased; this may be due to the better detection and treatment of the underlying infection or improved supportive care. Nevertheless, it is estimated that the rate of death from severe sepsis ranges from 30 to 50 percent despite advances in critical care.

In general and especially during a severe sepsis, the inflammatory and procoagulant host responses to infection are closely related. Inflammatory cytokines, including tumour necrosis factor α, interleukin-1β and interleukin-6 are capable of activating coagulation, whereas the procoagulant thrombin is capable of stimulating multiple inflammatory pathways. This may result in diffuse endovascular injury, microvascular thrombosis, organ ischaemia, multiorgan dysfunction and lead to death.

Activated protein C is an important modulator of the coagulation and inflammation associated with severe sepsis. Activated protein C is converted from its inactive precursor, protein C, by thrombin coupled to thrombomodulin. Activated Protein C modulates the systemic response to infection via three different mechanisms. Firstly, it exerts an antithrombotic effect by inactivating factors Va and VIIIa consequently limiting the generation of thrombin. Secondly, it indirectly increases the fibrinolytic response by inhibiting the plasminogen-activator inhibitor 1 (PAI-1). PAI-1 is a potent inhibitor of tissue plasminogen activator, the endogenous pathway for lysing a fibrin clot. Finally, as a result of decreased thrombin levels induced by rhAPC, the inflammatory response induced by thrombin is reduced. The conversion of protein C to activated protein C may be impaired during sepsis as a result of the down-regulation of thrombomodulin by inflammatory cytokines. Thus, reduced levels of protein C are found in the majority of patients with sepsis and are associated with an increased risk of death.

Drotrecogin alfa (activated) is a recombinant human Activated Protein C (rhAPC) which has an identical primary sequence to the endogenous human plasma-derived Activated Protein C. rhAPC has similar properties to those of endogenous human activated protein C. It is an active substance, which has been developed for the treatment of adult patients with severe sepsis with multiple organ failure when added to best standard care (i.e. severe sepsis).

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

Xigris is supplied as a powder for solution for intravenous infusion. It is formulated in 5 mg and 20 mg presentation. The active substance contains a recombinant form of human Activated C Protein (rhAPC).

The detailed composition of both presentations is given in the table below

Ingredient	Quantity per Vial	Quantity per Vial	Function
Active Ingredient			
recombinant human Activated Protein C	20 mg	5 mg	Active Ingredient
Other Ingredients			
Sodium Chloride	152 mg	38	Bulking Agent/ Solution-State Stabiliser
Citrate [1]	30.2 mg	7.56 mg	Buffering Agent
Sucrose	120 mg	30 mg	Bulking Agent/ solid-state Stabiliser

[1] The source of citrate (citrate ion) is a buffer system composed of Citric Acid, Ph.Eur., USP, Sodium Citrate, Ph.Eur., USP, hydrochloric acid, and sodium hydroxide. The source of Citric Acid, Ph.Eur., USP is the rhAPC Drug Substance.

The 5 mg and 20 mg presentations are prepared using the same drug product manufacturing solution and thus contain the same excipients at the same relative ratios. The two strengths are prepared by varying the amount of filling solution for lyophilisation to yield 5 mg and 20 mg rhAPC. After reconstitution the solution exhibits the same strength with respect to all ingredients of the medicinal product.

In order to assure the nominal dose delivery, a vial of the proposed commercial product, 5mg will typically contain a 6 % excess (5.30mg rhAPC/vial) and a vial of the proposed commercial 20 mg presentation will typically contain an overfill of 4.05% (20.81 mg rhAPC/vial).

Reconstitution studies have been performed with regard to dissolution, stability, microbial quality and compatibility with different dispensing material.

Container
The container is the same for both presentations: an ammonium sulfate treated type I glass vial of 5 ml (5 mg rhAPC) and 20 ml (20 mg rhAPC) closed with butyl rubber stoppers and sealed with aluminium seals with flip caps. The container closure systems are adequately described and meet the Ph. Eur. Standards for use with parenteral products.

Clinical trial formulae
The used clinical trial formulae are well described.

Production and control of starting materials

Specifications
Tests and specifications for rhAPC drug substance have been established in accordance with the nomenclature and principles described in the ICH (Q6B) guidance document "Specifications, Test Procedures, and Acceptance Criteria for Biotechnology and Biological Products." The specification limits for rhAPC Drug Substance were established based on experience with the manufacture of this product by Eli Lilly and Company and Lonza Biologics Inc. In particular, they were established based on extensive characterization of the rhAPC reference standard, routine testing and additional characterization of clinical trial lots and full-scale consistency lots, stability studies, and analytical methods validation

The proposed release specifications are considered adequate to ensure the quality of the produced active substance batches.

Development genetics
Gene isolations strategy and rationale
The cDNA used for the generation of the production cell line for Lilly rhAPC [drotrecogin alfa (activated)] was derived from the liver of a healthy male donor organ. Since the liver is the primary biosynthetic source of circulating plasma Protein C, it was expected to be the best source for mRNA. Part of the nucleotide sequence of the plasmid includes the coding for a preproprotein of 461 amino acids, which corresponds to the complete coding sequence of the human Protein C gene with short flanking sequences.

Description of the host cell line
The human cell line, HEK293, into which the Protein C expression vectors were introduced, was purchased from American Tissue Culture Collection (ATCC). HEK293 from ATCC was transformed with adenovirus 5 DNA. The Ad5 DNA originally used to transform the cell line is classified as a non-oncogenic (Group C) adenovirus.

The rationale for the use of the cell line for the production of recombinant human Protein C is based on the ability of this cell to correctly perform a series of complex post-translational modifications that are required for functional activity (see below).

Preparation of the production cell line
Construction of the expression vector
The construction of the final expression vectors with all intermediary steps is extensively described. The transcription of the human Protein C has been described adequately.

A restriction/function map of the plasmid pGTC is shown in the dossier and the details of origin and sites of the exact fragments contained in the vector have also been presented.

Selection and cloning of production cell line
The generation of the production line was accomplished by first isolating an initial Protein C-producing line in order to produce a clone, capable of higher production of human recombinant Protein C. This clone was subcloned to obtain a single cell isolate which was further used to generate the initial certified Master Cell Bank (MCB). The Manufacturer Working Cell Bank (WCB) was then generated from the MCB.

Description of the cell line
The initial cell line was extensively tested and found to be negative for the presence of human and nonhuman adventitious agents.

Preparation and testing of cells at the limit of in-vitro age for production
A number of tests were achieved in order to demonstrate the stability of the expression construct for rhPC. The evaluation of the stability of the expression construct is based on comparisons of the tests results from the MCB, the WCB and the cells at or beyond the limit of *in-vitro* age of production.

In general, the manufacturer has achieved a clear description of the whole genetic development, including plasmids constructions and cell lines isolation, that allows understanding of the way for the final isolation of the producing cell line.

Cell Bank System
The whole cell bank system is adequately described. Characterization testing of all cell banks has been performed in accordance with ICH Guideline Q5D and study reports are included. The testing protocol for new WCB is fully documented and considered to be adequate.

Production
The applicant has provided a flow chart with a general overview of the rhAPC active substance production.

Fermentation and harvesting
Cell culture production of the recombinant human protein C (rhPC) zymogen (non-active precursor of rhAPC) is carried out in a bioreactor.
The applicant has provided flow charts illustrating the steps of the process, as well as Critical Control Parameters and Criteria for Forward Processing.

The overall production process, batch description and raw materials (culture media) are well documented. In-process tests and criteria for forward processing are adequate. Process streams which fail to meet forward processing criteria will not be reprocessed.

Purification
The viral inactivated rhPC raw material is concentrated and purified using a pseudo-affinity anion-exchange capture chromatography step. This step results in a high degree of purification. Capture mainstreams are combined to provide an appropriate quantity of rhPC for downstream purification. The zymogen is then converted to rhAPC by batch activation with bovine thrombin. During the assessment, concerns on the bovine origin of thrombin and its purity with regards Factor V and Va were raised to the applicant. Reassuring information on the quality of drug substance ass well as on the bovine thrombin raw material was provided.
The activated material is subjected to removal of any potential contaminating viruses. Additional purification is performed. Updated information on the final freezing step before storage and shipping has been adequately documented.

Characterization of the drug substance
Recombinant human Activated Protein C (rhAPC) is a 2-chain glycoprotein containing four N-glycosylation sites and 12 disulfide bonds. The heavy chain contains 250 amino acids, in which seven residues are cysteine and three N-linked glycosylation sites (Asn-248, -313, and -329). The seven cysteine residues form three disulfide bonds within the heavy chain and one disulfide bond between the chains. The light chain contains one N-linked glycosylation site (Asn-97) and 17 cysteine residues, which form eight disulfide bonds within the light chain and one disulfide bond between the chains. The first nine glutamic acids on the light chain are γ-carboxylated (Gla) and aspartic acid 71 is β-hydroxylated. rhAPC has an identical amino acid sequence as human plasma-derived Activated Protein C, but differs from the latter in its glycosylation pattern.

The rhAPC active substance is a frozen aqueous solution of rhAPC as well as citrate buffer and sodium chloride.

Full characterization of the active drug substance has been performed on a primary in house reference standard (RS0289), which was derived from rhAPC produced using the commercial cell bank and manufacturing process.
The structure of rhAPC has been established through various physicochemical techniques.
The structures of six rhAPC full-scale consistency lots were characterized and compared to the standard. Physico-chemical characteristics, amino acid/carbohydrate primary structure, secondary and tertiary structure of rhAPC are adequately documented.
The experimental data submitted for the full scale lots provide a good indication of a consistent production with regard to the structural integrity of the rhAPC drug substance.

Analytical development
rhAPC specific methods were developed at Eli Lilly and transferred to Lonza Biologics.
Validation of all product specific analytical methods is described in detail, the reference standards used are fully documented.

Process validation
Validation data are provided for validation of the criteria for Forward Processing and the Critical Process Parameters for each step of the production process. Several full scale commercial batches were included in the validation studies. Additionally, specification testing has been performed on 10 full scale commercial batches. The data provided demonstrate a good consistency of production.

Comparison of pilot-scale and commercial processes
During the clinical development program several process modifications were implemented. Preclinical as well as Phase 1 and 2 clinical trial lots were manufactured using the first development process. Subsequent optimization studies led to the development of the initial commercial process which was used to manufacture lots for Phase 3 clinical trials. The latter process provided improved stability for the drug substance manufacturing solution. In changing from one to the other process a step was added to provide a greater level of viral safety assurance and certain animal-source raw materials were removed. Finally and during Phase 3 a slightly modified commercial process was introduced. This later one used a new master and working cell bank (cloned from the existing working cell bank) and the form of the drug substance was changed. In addition, the citrate concentration in the final elution step was reduced from 20mM to 10mM.

A summary of the range of assay values obtained for pilot-scale lots produced by the three mentioned manufacturing processes have been provided by the applicant. Although, variability for some parameters tended to be considerably greater for the first process, in general, the assay values observed for the first processed material were comparable to those observed using the later processes.

The applicant has performed extensive comparability studies between lots produced by the different processes used throughout the development of the drug substance production. The data provided show that the full-scale drug substance lots meet the proposed specifications, and they give a good indication of comparability between full-scale and pilot-scale lots. In addition to the specification assays, a diverse battery of additional characterization assays were performed to assess structural integrity and purity. It is also important to note that commercial scale lots were used in late phase clinical trials.

Impurities
The data presented by the applicant demonstrate the capacity of the purification process to effectively remove product as well as process related impurities.

Batch results
Six full scale batches of rhAPC active substance were tested. In general, the data provided show a good consistency of production with regard to potency, integrity and purity.

Excipients

All excipients used are described in either the Ph. Eur or the USP, and the rationale for the choices of the used monograph are justified.

Packaging material

All used packaging material is fully described, and complies with the standards of the Ph. Eur. Container and closure systems integrity have been thoroughly investigated, especially with regard to drug substance adsorption/absorption, and material leakage.

Finished Product

Development Pharmaceutics
The development pharmaceutics (choice of excipients, freeze drying process, final container, overfill) for the rhAPC final product are adequately described, and provide a sound basis for a good final product quality.

Method of preparation
The active ingredient, *rhAPC drug substance frozen solution*, produced at Lonza Biologics, New Hampshire, US, is transferred to the Final Product manufacturing site where formulation and filling of the dosage forms is done. Packaging and labelling for the EU will be conducted at Lilly Pharma, Germany. EU Release testing will be performed at Eli Lilly, France.

Manufacturing process
A flowchart of the whole final product manufacturing process (from formulation to packaging) as well as the list of performed in-process controls have been provided.
In general, the manufacturing process and the used equipment is well described.

During the assessment, a product related inspection was requested by the CPMP for two manufacturing sites, the active ingredient and the manufacturer of the finished product.
A re-inspection was requested in order to verify that the implementation of the action plan proposed by the manufacturer (i.e. resolution of GMP related deficiencies, the evaluation of results of the PQ-batches, the completion of the shipment validation). The inspection took place in March 2002 and the outcome from the inspection report was favourable and in compliance with the Community Good Manufacturing Practice requirements.

Validation of final product manufacturing process
The applicant has provided validation data of the active substance for three full-scale lots of each drug product presentation produced at the commercial manufacturing facility. Product quality for the full-scale lots was comparable to that of the clinical lots produced at pilot-scale.
The data presented give a good indication that the final product manufacturing process is reproducible and adequately controlled. Validation studies data demonstrate a good consistency with regard to the quality of the rhAPC active substance, as well as within different production batches.

Specifications and routine tests
The release specification limits were established based on experience with the manufacture of this product.

Stability

Active substance
The company has provided updated drug substance stability data for three pilot-scale and seven full scale batches when stored frozen for up to 24 months. The data provided indicate a good stability of the drug substance. Based on the available data, the shelf life proposed can be granted.

Final Product
Based on the stability data provided in the dossier, the proposed 24 months shelf life at 2-8°C is accepted.

Reconstituted Final Product
The data submitted by the company support the proposed holding time of 3 hours for the reconstituted Final Product.

RhAPC Intravenous Solution
Upon use, the reconstituted drug product is further diluted with sterile 0.9% NaCl solution prior to continuous I.V administration.
Studies have been conducted to assess the in-use stability of I.V. solutions of diluted rhAPC Drug Product with readily available and commonly used European infusion sets.
The SPC has been adapted accordingly, with a statement on the types of materials for NaCl 0.9% solutions and infusion sets, more specifically concerning the materials (PVC, PE, PP), which have been proved to be compatible with the drug product.

Virological documentation
The producing human cell line has been adequately controlled for contamination with human viruses. Fermentation of the cell line requires several additives of bovine origin and activation of rhPC during the manufacture is achieved by the use of bovine thrombin. Therefore, each lot of additives as well as the producing cell culture at the end of each fermentation run is tested for adventitious viruses. Polyoma virus is not included in routine testing. However, the production of the bovine-derived substances include steps which might have some capacity to remove or inactivate viruses. A complete validation of these steps on their capacity to inactivate/remove polyoma viruses has been announced. The manufacturing and purification steps of the active substance contribute to the viral safety although complete validation with regard to clearance of bovine polyoma virus is still on-going.

TSE documentation
Fetal calf serum used at the MCB and WCB level as well as bovine derived substances which are used during the fermentation process and the bovine thrombin used for activation of rhPC are covered by EDQM certificates and do not raise concerns. Recombinant human-insulin used during the fermentation process of rhPC is derived using a WCB free of ruminant materials. TSE safety has been demonstrated sufficiently.

Discussion on chemical, pharmaceutical and biological aspects

Xigris is supplied as powder for solution for infusion in two presentations : 20 mg and 5 mg. The active substance, recombinant human Activated Protein C (rhAPC), is obtained by DNA recombinant technology. An inactive zymogen (rhPC), produced by a well characterised primary human cell line (HEK293), is subsequently activated by addition of bovine thrombin, followed by several purification steps before storage or formulation of the drug product.

Issues that remained unsolved at the time of the December 2001 CPMP meeting have all been addressed properly in the last responses of the applicant:

An appropriate potency (APTT) assay has been developed and validated in accordance with Ph. Eur requirements;
All available drug substance and drug product batches have been retested with this new assay, and the submitted results provide assurance on product quality and production consistency, also with regard to the filling strategy proposed by the company. The former has been further confirmed by pre-authorisation sample testing;
In use stability has been checked for commonly available commercial EU sources of infusion sets, and those compatible with the product are mentioned in the SPC.
Additional measures have been taken to ensure the absence of residual bovine thrombin impurities.
The requested information regarding product identity specifications, including justification of specification limits (RP-HPLC, Peptide mapping), has been provided.

In summary, as the application stands now, the chemical, pharmaceutical and biological documentation is of an acceptable quality, and provides a sound basis for the production of a well controlled, consistent product, provided the applicant agrees to the list of commitments issued by the CPMP.

3. Part III: Toxico-pharmacological aspects

Pharmacodynamics

Drotrecogin alfa (activated) is a recombinant human Activated Protein C. The activated protein C is a protease belonging to the serine proteases family. Protein C (and activated protein C) plays a major role in the regulation of coagulation. This activity is enhanced by the non-enzymatic protein cofactor, Protein S. The basis for the antithrombotic function of activated protein C is its ability to inhibit thrombin function. In addition, activated protein C is an important modulator of inflammation associated with severe sepsis.

Part of the pre-clinical studies (single-dose, repeat-dose, vascular irritation, fertility, and teratology studies) were conducted with TCC-222, a recombinant activated Protein C product similar to rhAPC in carbohydrate structure, primary protein structure, and biological activity. However, different processes are used to produce and purify these two recombinant proteins, and the final drug products are formulated in different buffer systems. Physiochemical differences have been observed between TCC-222 and rhAPC, but it is not known if these differences are clinically relevant. No comparability studies have been performed on TCC-222 and rhAPC.

- Antithrombotic activity

Activated protein C inhibits the formation of thrombin. Protein C activation occurs *in vivo* on the surface of intact endothelial cells by the catalytic complex of thrombin and thrombomodulin, an integral membrane protein. Activated protein C specifically acts as an antithrombotic agent by hydrolysing factors Va and VIIIa. Like most of the other factors regulating haemostasis, activated protein C functions as part of a large multi-protein complex on the endothelial membrane surface. The activity of activated protein C is inhibited by endogenous serine protease inhibitors (serpins). These include protein C inhibitor, α_1-antitrypsin, α_2-macroglobulin, α_2-antiplasmin, and plasminogen activator inhibitor. Endogenous serine protease inhibitors are natural inhibitors for activated protein C, resulting in activated protein C having a very short circulatory activity half-life (less than 30 minutes) *in vivo*.

Antithrombotic and anticoagulant activity of rhAPC was investigated in a guinea pig arteriovenous shunt thrombosis model. Concentration dependent increments in activated partial thromboplastin time were observed *in vitro* in human and guinea pig blood.

RhAPC did not cause a depletion of FV or FVIII. The C_{ss} reached in clinical therapeutic conditions is about 50 ng/ml (well below 2 µg/ml). In the animal models tested, rhAPC exerted antithrombotic properties at doses that did not increase the bleeding times.

- Profibrinolytic property

The activated protein C exerts its fibrinolytic activity indirectly via enhanced endogenous fibrinolytic enzyme activities of tissue plasminogen activator and urokinase. Activated protein C enhances this activity by inhibiting plasminogen activator inhibitor-1 (PAI-1) and preventing the activation of thrombin activatable fibrinolysis inhibitor (TAFI). The dose-dependent inhibition of plasminogen activator inhibitor-1 activity by rhAPC is observed at concentration of 1-30 µg /ml, well above therapeutic level. The results indicate that rhAPC inhibited plasminogen activator inhibitor-1 activity in a concentration dependant manner, similar inhibition caused by argatroban and Heparin was not observed. It was concluded that rhAPC has profibrinolytic activity that is mediated by plasminogen activator inhibitor-1 inactivation.

- Antiinflammatory properties

While *in vitro* studies suggest a direct anti-inflammatory role for activated protein C, *in vivo* responses in anti-ischaemic and anti-inflammatory animal models have not been consistent. In the rat, the anti-inflammatory properties of rhAPC are observed at doses ranging from 1 to 20 mg/kg (single injection IV), well above therapeutic level. The clinical relevance of the direct anti-inflammatory activity reported for activated protein C therefore remains unclear.

- Activity in animal models of sepsis

The effect of rhAPC on Baboon Response to LD_{100} *Escherichia coli* was evaluated. This study was performed to determine whether rhAPC co-infused with LD_{100} *Escherichia coli* would inhibit the disseminated intravascular coagulation (DIC) and lethal inflammatory responses. Eleven baboons receiving LD_{100} *Escherichia coli* were treated with (plasma derived) activated protein C. Five animals received 40-60mg total activated protein C prophylactically and six received the same amount of activated protein C therapeutically; two baboons were used to investigate the dose dependent

anticoagulant effects of activated protein C. The findings showed that the consumption of fibrinogen (DIC) was inhibited in 10 of 11 animals. Four of these 11 baboons were protected from lethal effects of E.coli.. While some of the 11 animals survived, the data suggested that rhAPC may be effective in this baboon model of gram-negative bacteremia. However, because of the small sample size, with a single type of gram-negative bacteria used, and with different dosing regimens used, it is difficult to predict whether rhAPC may be beneficial in human patients with severe sepsis.

- Safety pharmacology

Since activated protein C is a potent antithrombotic, bleeding might be a potential pharmacological toxicity. This was confirmed by the literature and the pharmacology and toxicology studies performed. Beyond this, no direct vasoactive effects of rhAPC were demonstrated. In several studies, bleeding was generally limited to surgical sites and was not considered to be substantive.

- Pharmacodynamic interactions

In vitro studies performed to evaluate the potential interaction between rhAPC and unfractionated heparin and low molecular weight heparin indicated possible effects on the pharmacokinetic and pharmacodynamic properties of rhAPC. The effects of unfractionated heparin and low molecular weight heparin on the activity half-life of rhAPC were also evaluated *in vitro* in plasma, utilising therapeutic concentrations of unfractionated heparin and low molecular weight heparin (below 1 U/ml) and rhAPC. No significant shortening or effect on the activity half-life of rhAPC was observed; neither with unfractionated heparin nor low molecular weight heparin. There was however an additive effect of unfractionated heparin and activated protein C on the prolongation of *in vitro* activated partial thromboplastin clotting time. This observation was reproduced *in vitro* with rhAPC. The additive effect of unfractionated heparin and APC on the prolongation of in vitro APTT was shown by the applicant to be almost entirely mediated by antithrombin III. The conclusion of that *in vitro* assay suggests that increased APTT as a result of heparin/rhAPC synergy might be a concern even at reduced AT-III (up to 25% of normal) levels well below the 59% of normal median baseline level of antithrombin III found in severe sepsis patients. It would be very difficult to adequately address the question of potential additive effects of heparin and APC in animal models of sepsis. This is therefore discussed further in the clinical part of this report.
Unlike unfractionated heparin, low molecular weight heparin at therapeutic concentrations did not show any synergistic effect on the prolongation of activated partial thromboplastin clotting time by rhAPC.
In order to evaluate the potential of rhAPC to interact with aspirin, rhAPC was studied in a guinea pig thrombotic model with or without aspirin and other antiplatelet compounds. RhAPC was found to be synergistic with aspirin in antithrombotic activity without producing any significant increase in template bleeding time or any observed increase in bleeding.

Pharmacokinetics

Pharmacokinetic properties of drotrecogin alfa activated were investigated in *cynomolgus, rhesus* monkeys and rodents.

- Pharmacokinetic after a single dose administration

RhAPC was given to male *cynomolgus* monkeys as a single intravenous bolus dose of 0.1 mg/kg. The average peak plasma concentration was observed 5 minutes after the administration and was 956 ng/mL. Plasma concentrations declined rapidly below 10 ng/mL between 1.5 and 3 hours after the administration. The terminal phase half-life was estimated to be approximately equal to 20 minutes.

A study evaluated the distribution of rhAPC following a single administration in mice. Tissue distribution of radiolabelled rhAPC was investigated in mice after a single dose of 0,2 mg/kg. The highest concentration was seen in the liver 5 minutes after the injection. Further, radioactivity levels increased at 24 and 168 hours after administration suggesting that metabolites containing ^{125}I would be incorporated into macromolecules in the animal model investigated. In addition, several

published reports have examined the tissue distribution, metabolism and elimination of plasma-derived activated Protein C in the mouse, guinea pig, and rabbit proteases. Overall, the data from these studies are consistent with the clearance of activated Protein C from the circulation being mediated by a combination of at least three processes including the inhibition of the enzymatic activity of activated protein C by endogenous protease inhibitors, the clearance of activated protein C and/or activated protein C-serine protease inhibitor complexes by organs such as liver and kidney, and the degradation of activated protein C and/or activated protein C-serine protease inhibitor complexes by circulating or tissue proteases.

- Pharmacokinetics after administration of repeated doses

RhAPC was administered to male *rhesus* monkeys at a constant rate intravenously. Average concentrations at steady state (C_{ss}) were measured after administration of 1, 2, or 4 mg/m²/hr. At all dose levels, steady-state was achieved at 24 hours and levels were maintained through 96 hours. The average C_{ss} increased in a dose-related although not strictly dose-proportional fashion. Average steady-state concentrations were also measured after administration of 4.2, 8.3, and 16.7 mg/kg/day. There was a trend towards an increase in volume of distribution and a decrease in plasma clearance with increasing dose. The lack of proportionality was most evident between the 8.3 and 16.7 mg/kg/d dose levels. Since a major mechanism involved in the clearance of Activated Protein C is through its inactivation via interaction with circulating plasma protease inhibitors, the disproportionately high C_{SS} and AUC (that is, decreased clearance) at the high dose(s) may be related to saturation of the inhibitor-complex compartment leading to a higher than anticipated measurement of amidolytic activity.

A comparative study of pharmacokinetic parameters of rhAPC and plasma derived activated protein C was carried out in *cynomolgus* monkeys following intravenous administration. The half-life of the two test articles was shown to be different in the initial period after administration. The disappearance of rhAPC was found to be faster than that of plasma derived activated protein C. The half-life of rhAPC was reported to be slightly shorter than that of plasma derived activated protein C in a baboon AV shunt model. RhAPC has unique carbohydrate chains that may cause differences in the pharmacokinetics of rhAPC and plasma derived activated protein C.

Toxicology

- Single dose toxicity.

The single-dose toxicity program of rhAPC was conducted in the mouse and monkey. A single dose toxicity study was performed in ICR mice that were allocated to seven groups, two control groups and 5 groups of mice that received 0.78, 3.13, 12.5, 50 and 200 mg/kg IV of rhAPC respectively. Prolongation of haemostasis at the injection site was seen in animals that received 12.5 mg/kg of product or more. Necropsy revealed no macroscopic changes considered to be associated with the administration. No deaths occurred during the observation period and the minimal lethal dose was therefore estimated to be greater than 200 mg/kg bodyweight.

The potential toxicity of rhAPC was investigated in *cynomolgus* monkeys following a single intravenous administration at doses of 25 mg/kg and 100 mg/kg. One control group received Tris acetic acid buffer through the intravenous route. All animals in the treatment groups exhibited decreased movement, subcutaneous haemorrhage and swelling of the inner site of the thigh. These signs were seen immediately or within 60 minutes after administration. Subsequently, death occurred in both animals in the 25 mg/kg group and in one animal in the 100 mg/kg group at six hours after delivery. The remaining animal in the high dose group died the following day. Haematological investigations after administration revealed markedly prolonged prothrombin and activated prothrombin times. The pathological findings were subcutaneous bleeding extending from the inguinal to femoral regions, haemorrhage was also seen under the retroperitoneum. The cause of death was considered to be related to the loss of a large quantity of blood. Based on these findings the lethal dose was considered to be below 25 mg/kg.

- Repeated dose toxicity

The repeated dose toxicity of drotrecogin alfa activated was investigated in rhesus monkeys in a single 96-hour continuous intravenous infusion study and in cynomolgus monkeys in two studies (2-week and 1-month). It was concluded that the responsiveness of *rhesus* and *cynomolgus* monkeys to rhAPC utilised in the toxicology studies was comparable to that seen in the baboon in the pharmacodynamic studies.

A 96-hour toxicity study with rhAPC administered by continuous intravenous infusion was conducted in *rhesus* monkeys. The purpose of this study was to evaluate the toxicity of rhAPC when administered at doses of 0, 4.2, 8.3 and 16.7 mg/kg/day (approximately 0, 2, 4 and 8 mg/m^2/h) with an infusion rate of 1ml/kg/h through a catheter for 96 hours.

Two animals out of 4 in the high dose group died following a haemorrhage. As expected, infusion of rhAPC in all treatment groups resulted in increases in coagulation parameters. Dose-related increases in prothrombin time, activated partial thromboplastin time and whole blood partial thromboplastin time were measured throughout the dosing period. Moderate to severe haemorrhagic lesions affected the animals in the mid and high dose groups. However, haemorrhagic effects were not distinguishable between control and low dose group. In addition, there was a large intra- and inter-group variation in bleeding time. Based on these findings it was concluded that the no observable adverse effect level (NOAEL) was estimated to be 2 mg/m^2/hr.

RhAPC was administered intravenously to *cynomolgus* monkeys at doses of 0, 0.2, 1 and 5 mg/kg/day for two weeks. Haematological changes attributable to the pharmacological action of rhAPC were noted, including prolonged prothrombin time in the mid- and highdose groups and prolonged activated partial thromboplastin time in all dose groups 15 min after the end of administration. Although these effects were still present 180 minutes after dosing, full recovery was achieved by the next day. There were no adverse effects related to the repeated dosing. Although there was a decrease in erythrocytic parameters and an increased reticulocyte count, these were considered as being unrelated to rhAPC. Further, a decreased food intake was shown, but without any effect on body weight, in all treatment groups especially in the late stages of dosing. There were no treatment-related changes in ophthalmology, urinalysis, blood chemistry, occult blood, organ weight, and gross pathology. Consequently, since the observed changes could be attributed to the pharmacological action of rhAPC, 5 mg/kg/day was considered as the non-toxic dose (NOAEL).

TCC-222 was administered intravenously to monkeys at the same dose levels as in the previous study over a 1-month period. In general, higher doses produced prolongation of prothrombin time and activated partial thromboplastin time and haemorrhaging at sites of vascular injury, occasionally leading to morbidity and death. Effects at lower doses were limited to prolongation of activated partial thromboplastin time but without evidence of haemorrhaging. In addition, an intravenous dose toxicity study of rhAPC was performed in Monkeys with a 1-Month Recovery Period. RhAPC was administered intravenously to cynomolgus monkeys at doses of 0, 0.2, 1 and 5 mg/kg/day over a 4 weeks period coupled to a 4-week recovery phase to assess the effects of the test article as well as the reversibility of these effects. The haematological examination revealed prolonged prothrombin time in all animals in the mid- and highdose group. Prolonged activated partial thromboplastin time in all treatment groups was noted 15 minutes after the end of dosing. These effects were seen to be reversible at the end of the recovery period. Histopathological examination revealed some abnormalities of megakaryocytes in all treatment groups. These changes disappeared during the recovery period except for those in megakaryocytes.

In conclusion, changes observed in monkeys at the same level or in small excess of the human exposure during repeated dose studies, were all related to the pharmacological effect of rhAPC and include, beside the expected prolongation of APTT, decreases in haemoglobin, erythrocytes, and haematocrit, and increases in reticulocyte count and PT.

- Reproduction toxicity

A study to investigate fertility and reproductive performance was conducted in female rats. The purpose of this study was to investigate any adverse effect of TCC-222 on mating, fertility and

early embryonic development when administered intravenously to female rats on a daily basis from 14 days prior to mating, throughout the mating period until day 7 of gestation in doses from 0.6, 2 and 5 mg/kg/day.

There were no signs of influence on reproductive functions and general toxicity in female rats. The effects on embryonic and foetal development were investigated in rats following intravenous administration of TCC-222. The study was performed to investigate the influence of TCC-222 on pregnant rats and on development of embryos/foetuses when administered intravenously in doses of 0, 0.6, 2 and 5 mg/kg/day to female rats from implantation to closure of the hard palate (day 7-17 of gestation). No deaths or abortions were observed in this study.

There were no significant differences between the control and treatment group regarding parameters such as number of corpora lutea or implantation sites, pre- and post-implantation loss, number of live foetuses, sex ratio and foetal weight.

Since the animal studies performed with respect to effects on pregnancy, embryonal/foetal development, parturition and postnatal development are very limited, the potential risk for humans is considered unknown. APC should therefore not be used during pregnancy unless clearly necessary.
Furthermore, it is not known whether the product is excreted in human milk or if there is a potential effect on the nursed infant. Therefore, the patient should not breast feed whilst treated with Xigris. This has been adequately reflected in the SPC.

- Genotoxicity studies

The mutagenic potential of rhAPC was evaluated in an *in vitro* chromosomal aberration test using human peripheral lymphocytes. It was shown that the test article did not induce any structural aberrations but a few numerical aberrations at high concentration in cultures treated for 48 hours. These results indicate that the test article was not clastogenic under the test conditions and that the polyploid induction might be due to the non-specific reaction by the treatment within high concentration in the presence of cytotoxicity.

The *micronucleus* test for rhAPC was performed in CD-1 (ICR) mice following intravenous injection. In this study the ability of rhAPC to induce *micronuclei* in mice bone marrow erythrocytes was investigated after a single intravenous administration in male and female mice in doses of 25, 50 and 100 mg/kg. The analysis of bone marrow smear revealed no significant increase of micronucleated erythrocytes.

- Carcinogenicity studies

In view of the short duration of treatment and in accordance with current guidance documents, studies to assess the carcinogenic potential of rhAPC were not conducted.

- Local tolerance

Studies specifically designated to evaluate the local tolerance of the product were not conducted; however, no evidence of irritation was identified at the injection- or infusion sites in any of the repeated dose toxicity studies. Additionally, mixture of rhAPC with whole blood from *rhesus* monkeys or humans did not cause haemolysis.

- Immunogenicity studies

A pilot toxicity and immunogenicity study was performed in *rhesus* monkeys following an intravenous administration of drotrecogin alfa activated. *Rhesus* monkeys were given two intravenous infusions of rhAPC. Loading doses of 0, 16, or 80 mg/m^2 were given by intravenous bolus infusion and were followed by 48- or 72-hour continuous intravenous infusion of 0, 16 and 80 mg/m^2/hr.

Eight weeks later the same animals received a second continuous intravenous infusion of 0, 4, or 8 mg/m^2/hr over a 72-hour time period. Treated animals were thereafter observed for an additional 12 weeks.

Severe haemorrhagic anaemia, from bleeding at catheter and venipuncture sites, occurred following the first infusion in both the 16- and 80-mg/m^2/hr treatment groups; bleeding times were increased in the 80-mg/m^2/hr-treatment group. There was no evidence of anaemia or haemorrhage following the second infusion. Coagulation parameters (i.e. whole blood partial thromboplastin times, activated partial thromboplastin times, and prothrombin times) were increased in a dose-related manner following the initial treatment phase and again following re-challenge. One animal each in the 4- and 8-mg/m^2/hr treatment groups was judged moribund and euthanasied. Both euthanasied animals exhibited lesions consistent with disseminated intravascular coagulation secondary to septicemia.

Anti-APC IgG antibodies were produced following challenge and rechallenge with rhAPC; however, no adverse hypersensitivity reactions were associated with these nonneutralizing antibody responses.

In conclusion, the toxicity observed following treatment with rhAPC was considered an extension of the anticoagulant properties of the compound.

- Ecotoxicity/Environmental risk assessment

Given the physico-chemical properties of rhAPC and the predicted low concentrations likely to enter the environment, there was no need to carry out further investigations of this particular risk.

4. Part IV: Clinical aspects

Drotrecogin alfa is a recombinant human APC developed for treatment of patients with severe sepsis. APC is an important modulator of the systemic response to infection and has antithrombotic and profibrinolytic properties. The clinical efficacy of drotrecogin alfa activated has been evaluated in one dose-finding Phase 2 study (EVAA) and a Pivotal Phase 3 study (EVAD). The clinical studies were conducted in accordance with the principles of Good Clinical Practice.

Protocol	Phase	Design	Population	Duration
Study F1K-LC-GUAA/B: Dose-ranging: short infusion to steady state.	2 Phase 1 studies	Open-label	2 x 4 (healthy males)	3 single 3 hour IV perfusions per subject separated by 2 wks
Study F1K-LC-GUAC/D: Dose-ranging: 6 and 24-hour infusions.	2 Phase 1 studies	Open-label	32 subjects (24 completed the study) – 51 subjects (43 completed the study)	Two dosing periods 6- and 24- hr IV infusions separated by at least 2 wks (GUAC) and 5 days (GUAD)
Study F1K-LC-GUAE: Study in end stage renal disease.	Phase 1	Open-label	13 subjects (12 completed the study)	One 6-hr IV infusion

Study F1K-LC-GUAF: Aspirin interaction.	Phase 1	Part A: aspirin alone Part B: single blind crossover design	Phase 1: 15 subjects Phase 2: 27 subjects	2 phases: 1 single dose of aspirin then 2 treatments separated by at least 14 days (aspirin or placebo foll. by rhAPC)
Study F1K-LC-EVAK: Bolus injection study.	Phase 1	Open-label	11 subjects	(rhAPC administered either via a bolus or a 6-hr constant rate IV infusion)
Study F1K-LC-EVAM: Loading-dose: dose range.	Phase 1	Open-label	14 subjects	Single loading dose followed by a 5.5 hr constant rate IV infusion
Study F1K-LC-EVAB: activated protein C: dose ranging study in hereditary protein C deficient patients	Phase 1B	Open-label	9 patients (6 patients enrolled)	Up to 3 24-hr IV infusions; each infusion separated by a minimum of 3 wks
Study F1K-MC-EVAA: Phase 2 study to determine the safety, pharmacokinetics and effective dose range and dosing duration for rhAPC in severe sepsis.	Phase 2	Double blind, placebo controlled, dose ranging.	Stage 1: 72 patients Stage 2: 59 patients Total 135 enrolled. 131 intent to treat patients	Stage 1: 48-hr continuous IV infusion Stage 2:96-hr continuous IV infusion
Study F1K-MC-EVAD: A phase 3 study to determine the efficacy and safety for rhAPC in severe sepsis.	Phase 3	Double blind, placebo controlled	1728 patients 1690 intent to treat patients	96 $\pm$ 1-hr continuous IV infusion

Clinical pharmacology

The pharmacokinetic and pharmacodynamic features of drotrecogin alfa (activated) were studied in 8 Phase-1 studies involving 112 naive exposures to rhAPC in healthy subjects. These properties were further investigated in one phase II dose-finding study (EVAA) and in the pivotal phase III study (EVAD).

Pharmacodynamics

- Mechanism of action

The Phase 1 studies were limited to infusions of rhAPC at 0.5 to 50 µg/kg/hr for durations up to 24 hours. The dose-finding studies in healthy subjects revealed a good correlation between rhAPC infusion rate, plasma rhAPC concentration, and activated partial thromboplastin time prolongation.

Activated partial thromboplastin time had to be assayed immediately after blood sampling because of rapid inactivation of rhAPC by plasma serine-protease inhibitors (serpins - natural inhibitors of the proteases belonging to the family of the serine proteases). Activated partial thromboplastin time increased about 1.5-fold during 24-hr infusion of rhAPC at 24 µg/kg/hr, and about 2-fold at 48 µg/kg/hr. Prothrombin time and bleeding time were either slightly prolonged within the normal range, or remained unchanged. Factors V and VIII declined slightly while remaining within the normal range. Platelet function was unaffected.

Pharmacokinetics

- General:

Drotrecogin alfa (activated) and endogenous human Activated Protein C are inactivated in plasma by endogenous protease inhibitors but the mechanism by which they are cleared from plasma is unknown. Plasma concentrations of endogenous Activated Protein C in healthy subjects and patients with severe sepsis are usually below detection limits (< 10 ng/ml) and do not significantly influence the pharmacokinetic properties of drotrecogin alfa (activated).

The pharmacokinetics of drotrecogin alfa activated was characterised in patients with sepsis. Plasma concentrations of rhAPC were measured using an immunocapture–amidolytic activity assay specific for APC. This method does not distinguish between rhAPC and endogenous activated protein C, but since the plasma levels of the latter (~2 ng/mL in normal subjects) are much lower than those of administered rhAPC (~30–100 ng/mL at the therapeutic dose), this lack of specificity is not a concern.

No metabolisation study with rhAPC was performed since it is a short-acting human recombinant protein. Both endogenous APC and rhAPC are rapidly inactivated by the plasma serine proteases inhibitors (serpins). The mean plasma clearance of rhAPC in healthy subjects was 28 L/hr. Steady state was reached in less than 2 hours after starting the infusion. Two studies were performed to evaluate the benefit of loading doses (bolus or injections).

The volume of distribution of rhAPC at steady-state was 10 to 17 L in healthy subjects, close to the volume of extracellular fluid. The mean plasma concentration in healthy subjects at steady-state, based on all infusions given during Phase 1 studies, was 70 ng/mL for a 24-hr infusion at 24 µg/kg/hr. There was a strong linear relationship between infusion rate (0.5 to 50 µg/kg/hr) and average steady-state concentrations across all subjects. The half-life of rhAPC measured at the end of an infusion was relatively short (0.5 to 1.9 hr). The pattern of disappearance of rhAPC was biphasic, with a rapid initial phase ($t_{½\alpha}$ = 13 min) accounting for 80 % of the AUC and a slower second phase ($t_{½\beta}$ = 1.6 hr). In both healthy subjects and sepsis patients, plasma rhAPC concentrations fell below the detection limit (10 ng/mL) within 2 hr after termination of the infusion.
The mean steady-state plasma concentration of drotrecogin alfa (activated) in healthy subjects receiving 24 µg/kg/hr is 72 ng/ml.

In the Phase 3 trial, the pharmacokinetics of drotrecogin alfa (activated) was evaluated in 342 patients with severe sepsis administered a 96-hour continuous infusion at 24 µg/kg/hr. The pharmacokinetics of drotrecogin alfa (activated) were characterised by attainment of steady-state plasma concentration within 2 hours following the start of the infusion. In the majority of patients, measurements of Activated Protein C beyond 2 hours after termination of the infusion were below the quantifiable limit, suggesting rapid elimination of drotrecogin alfa (activated) from the systemic circulation. The plasma clearance of drotrecogin alfa (activated) is approximately 41.8 l/hr in sepsis patients as compared with 28.1 l/hr in healthy subjects.
In patients with severe sepsis, the plasma clearance of rhAPC was statistically significantly decreased by renal impairment and hepatic dysfunction, but the magnitude of the differences in clearance (<30%) does not warrant any dosage adjustment in patients with renal disease. Understandably, there was no specific study in patients with hepatic disease, since the risk of bleeding would be unacceptably high.
In addition, no dose adjustments are required in adult patients with severe sepsis with regard to age, gender, or hepatic function (as measured by transaminase levels). The pharmacokinetics of

drotrecogin alfa (activated) has not been studied in patients with severe sepsis and preexisting endstage renal disease and chronic hepatic disease. This is appropriately reflected in the SPC.

- Interaction studies:

Only one formal interaction study was performed evaluating the effect of a single dose of 500 mg enteric-coated aspirin given 24 hours prior to rhAPC. No effect of aspirin on rhAPC pharmacokinetics was detected under these admittedly restrictive conditions. Caution is required if rhAPC is to be infused shortly after, or during, administration of any drug that can modify coagulation parameters or decrease platelet aggregation.

Compared with healthy subjects, the pharmacokinetics of rhAPC in sepsis patients were characterized by a 50 % faster clearance. This did not modify the time to reach steady-state or the elimination half-life of rhAPC in a clinically significant way. However, a much larger variation in individual C_{ss} and C_{lp} was observed in sepsis patients (see discussion on pharmacokinetics).

Clinical efficacy

The clinical efficacy of drotrecogin alfa (activated) was investigated in a dose-finding phase II study and a phase III (pivotal) study.

Phase 2 dose-response study (F1K-MC-EVAA).

This was a multicentre (40 centres in total), double blind, randomised and placebo-controlled dose-ranging study. It was undertaken in order to characterise the safety, pharmacokinetics, and effective dose range and dosing duration for drotrecogin alfa (activated) (rhAPC) in patients with severe sepsis. Further, the purpose of the study was to determine a dose of rhAPC that would have an effect on the coagulation abnormalities associated with severe sepsis as well as a favourable safety profile.

The study was divided into 2 stages: during stage 1 doses of rhAPC were administered as a continuous intravenous infusion for 48 hours and during stage 2 doses were administered for 96 hours. The initial rhAPC dose used in both stages was 12 µg/kg/hr; the maximum dose used in Stage 1 was 30 µg/kg/hr and in Stage 2, 24 µg/kg/hr. The study was limited to adult patients of either gender with severe sepsis with a known or suspected site of infection that met at least three modified systemic inflammatory response syndrome criteria and at least one organ failure criterion. Intensive care unit patients of either gender were evaluated for entry into the study if they met Criterion A (modified systemic inflammatory response syndrome [SIRS] criteria) and Criterion B (organ failure) qualifications.

Dosing groups

Stage 1 (48 hours infusion)		Stage 2 (96 hours infusion)	
12 µg/kg/hr	11 patients	12 µg/kg/hr	14 patients
18 µg/kg/hr	11 patients	18 µg/kg/hr	15 patients
24 µg/kg/hr	12 patients	24 µg/kg/hr	15 patients
30 µg/kg/hr	12 patients		
Placebo	26 patients	Placebo	15 patients

Studies in healthy volunteers indicated an underlying linear correlation between Activated Protein C concentration and a rapidly performed bedside measurement of activated partial thromboplastin time. Therefore, rapid bedside activated partial thromboplastin time measurements were performed during dosing to assess if a particular dose of rhAPC was associated with unacceptable elevations in activated partial thromboplastin time and by inference, high activated protein C concentrations.

- Objectives of the study – endpoints

The primary objective of the study was to assess the safety of administration of rhAPC as a function of dose and dose duration; to determine the degree to which the coagulation abnormalities of severe sepsis are affected by the administration of rhAPC as a function of dose and dose duration; to determine for use in future studies, an effective dose and dose duration of rhAPC administration, based on the ability of rhAPC to alter the coagulation abnormalities of severe sepsis.

The primary coagulation markers used in this study were D-dimer (fibrin degradation products) level, fibrinogen level, and platelets count. All patients who were enrolled and received study drug for any length of time were included in the primary efficacy analyses.

The major surrogate marker for assessing the efficacy of rhAPC was D-dimer concentration. Data for prothrombin time and activated partial thromboplastin time were also collected and analysed. The prothrombin time reflects the activity of the extrinsic and common pathways of coagulation. The activated partial thromboplastin time explores the integrity of the intrinsic and common pathways of coagulation. Prolongation of the prothrombin time and/or activated partial thromboplastin time indicates an abnormality of the secondary haemostasis, which may be present in patients with disseminated intravascular coagulation or sepsis. Interleukine-6 (IL-6) levels were monitored in an attempt to assess rhAPC effect by dose and duration on an inflammatory cytokine as compared to placebo.

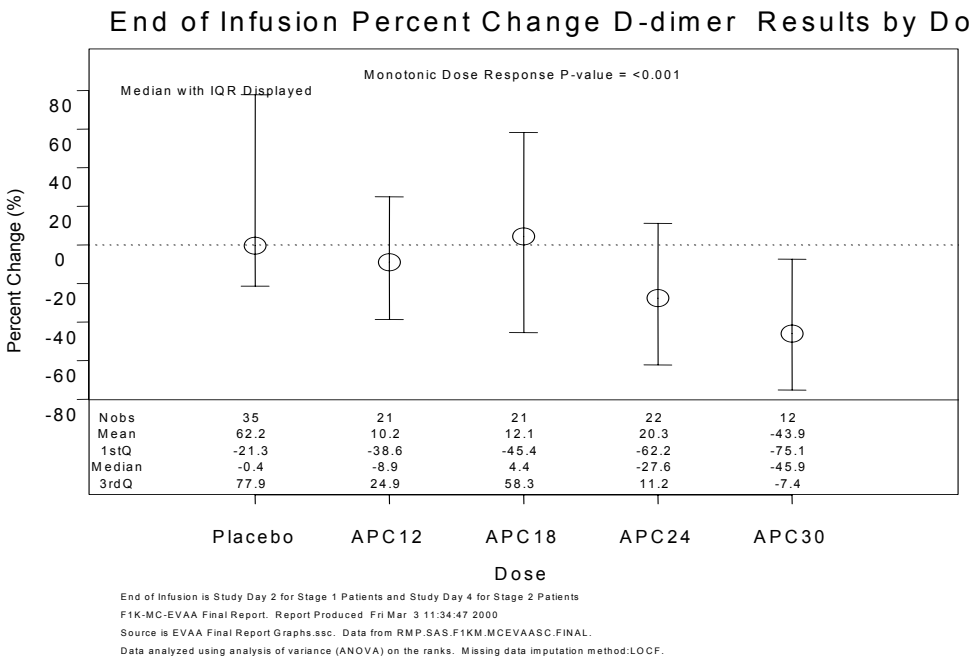
- Results

A total of 72 patients participated and received study medication in Stage 1 (rhAPC or placebo according to an allocation ratio of 2:1). A total of 59 patients participated and received study medication in Stage 2 (rhAPC or placebo in a 3:1 ratio). Patients were monitored for bedside activated partial thromboplastin time during infusions. If the bedside activated partial thromboplastin time was ≥95 seconds, the investigator reduced the infusion rate or stopped the infusion altogether.

Twenty-eight day survival was determined for all patients. A total of 64 (71.1%) of the 90 patients who received rhAPC and 27 (65.9%) of the 41 placebo patients survived the 28-day study period.

The results of this study indicate that a 96-hour continuous infusion provided greater benefit to the patient than a 48-hour infusion with no greater safety risk. During the study, the dose of 30 µg/kg/hour was associated with elevated levels of activated partial thromboplastin time that, in accordance with protocol requirements, necessitated a reduction in the dose of rhAPC. Considering the higher frequency of dose reductions recorded in the 30 µg/kg/hr for 48 hours dose group and the fact that 30 µg/kg/hr for 96 hours was not investigated, the highest dose assessed was 24 µg/kg/hr for 96 hours. Based on these results, a constant rate infusion of 24 µg/kg/hr of rhAPC for 96 hours was selected for a further Phase 3 study. Overall, the safety assessment of rhAPC compared with placebo demonstrated an acceptable safety profile for all doses and treatment durations.

The figure below contains a graphical presentation of the monotonic dose response with respect to the percent change from baseline D-dimers levels at the end of infusion ($p<.001$). The rhAPC doses ranged from 12 µg/kg/hr to 30 µg/kg/hr with 48-hour and 96-hour infusion duration's combined. The median percent decreases at end of the infusion were 0% for patients receiving placebo, 9%, for patients receiving 12 µg/kg/hr, 28% for patients receiving 24 µg/kg/hr, and 46% for patients receiving 30 µg/kg/hr. A 4% increase from baseline was observed in patients receiving 18 µg/kg/hr.



No statistically significant differences were observed in the percent change from baseline fibrinogen and platelet count levels for Stage 1 (48-hour infusion) patients and for Stage 2 (fibrinogen - 96-hour infusion) patients.

Baseline interleukine-6 data were available for 124 of the in total 131 patients (94.7%) who received the study drug. The observed distribution of baseline interleukine-6 values was similar among rhAPC-treated patients and placebo-treated patients. A statistically significant difference was observed among the five treatment groups at Study Day 1 and Study Day 3 (p=.04 and .02, respectively). The median percent decreases at Study Day 3 were 50%, 40%, 66%, 43%, and 93% for the placebo-treated, 12 µg/kg/hr for 48 hours rhAPC-treated, 18 µg/kg/hr for 48 hours rhAPC-treated, 24 µg/kg/hr for 48 hours rhAPC-treated, and 30 µg/kg/hr for 48 hours rhAPC-treated patients, respectively. The distributions of the percent change from baseline interleukine-6 measurements over time for all patients assigned to receive 96 hours of treatment in Stage 2 were also analysed, no significant differences among the four treatment groups could be demonstrated.

A total of 6 (4 rhAPC/2 placebo) bleeding events were reported as serious adverse events during the 28-day study period. Of the 4 events that occurred in rhAPC-treated patients, 1 event occurred in a patient assigned to receive 12 µg/kg/hr for 48 hours, 1 event occurred in a patient assigned to receive 18 µg/kg/hr for 48 hours, 1 event occurred in a patient assigned to receive 18 µg/kg/hr for 96 hours, and 1 event occurred in a patient assigned to receive 30 µg/kg/hr for 48 hours. None of these bleeding events occurred in patients with a bedside whole blood activated partial thromboplastin time ≥95 seconds. No bleeding events were noted in the 24 µg/kg/hr for 48-hour or 96-hour infusion duration.

The results of this study indicate that a 96-hour continuous infusion provided greater benefit to the patient than a 48-hour infusion with no greater safety risk. Based on these results, a constant rate infusion of 24 µg/kg/hr of rhAPC for 96 hours was chosen for further Phase 3 study.

Pivotal study (Pivotal Phase 3 study EVAD)

This was a double blind, placebo-controlled, multicentre (in total 164 centres in 11 countries), randomised Phase 3 study to evaluate the efficacy and safety for drotrecogin alfa (activated) in patients with severe sepsis.

The primary objective of this study was to compare the efficacy of rhAPC administration (24 µg/kg/hr administered as a continuous 96-hour intravenous infusion) to that of placebo on the primary endpoint 28-day all-cause mortality in patients with severe sepsis. RhAPC was added to best standard care, comprising adequate antibiotics, source control and supportive treatment (fluids, inotropes, vasopressors and support of failing organs, as required).

The secondary objectives of this study were to evaluate the effects of rhAPC on organ function (cardiovascular, respiratory, renal, hematology, and hepatic), to evaluate the health economic impact of rhAPC administration in patients with severe sepsis and finally to further characterize the pharmacokinetic properties of rhAPC administration.

Study population: The trial was designed to enroll 2280 patients. Eligible patients were at least 18 years old and had a diagnosis of severe sepsis, which was defined as meeting at least three modified systemic inflammatory response syndrome (SIRS) criteria, meeting at least one of five organ failure criteria (cardiovascular, renal, respiratory, hematology, or metabolic acidosis), and having a suspected or proven infection. Patients were randomly assigned to rhAPC or placebo according to a 1:1 ratio for the treatment allocation.
Exclusion criteria encompassed patients at high risk of bleeding, patients who were not expected to survive for 28 days due to a pre-existing, non-sepsis related medical condition, HIV positive patients whose most recent CD_4 count was $\leq 50/mm^3$, patients on chronic dialysis, and patients who had undergone bone marrow, lung, liver, pancreas or small bowel transplantation, and patients with acute clinical pancreatitis without a proven source of infection.
The inclusion and exclusion criteria of this phase 3 study are considered to appropriately define the target population for rhAPC treatment and has been reflected in the SPC.

Statistical analysis: Two interim analyses were planned, after 760 patients and 1520 patients had been enrolled (one-third and two-thirds of the planned study size, respectively). The two planned interim analyses occurred under the auspices of an independent Data and Safety Monitoring Board (DSMB). Statistical guidelines to suspend enrolment if drotrecogin alfa activated was found to be significantly more efficacious than placebo were determined *a priori* and used the O'Brien-Fleming spending function according to the method of Lan and DeMets. Trial enrolment was suspended after the second interim analysis [because the differences in the mortality rate between the two groups exceeded the *a priori* threshold for stopping the trial]. At the time when enrolment was suspended, 1728 patients had been enrolled in the trial, 1690 of these patients had received rhAPC (n = 850) or placebo (n = 840) for some length of time and constituted the intention-to-treat (ITT) population for the study.

The primary analysis was based on a Cochran-Mantel-Haenszel test in which the groups were stratified on the basis of three baseline covariates: severity of the disease as reflected by the score on the Acute Physiology and Chronic Health Evaluation II (APACHE II), age and plasma protein C activity level. The results from both stratified and nonstratified analysis are reported.

- Results

There was no statistically significant difference between the two treatment groups with respect to the duration of infusion, the proportion of patients who received the studydrug for ≥95 hours, or the proportion of patients who were treatment compliant. Per protocol, a patient was classified as treatment compliant if the patient received ≥90% of the intended 96-hour infusion. In the rhAPC treatment group, 654 (76.9%) patients received ≥95 hours of the study drug and 707 (83.1%) patients received either ≥95 hours of the study drug or died during the infusion or within 1 hour of study drug discontinuation.

Mortality analysis

Based on a nonstratified mortality analysis for the intent to treat (ITT) population, relative to the placebo treatment group, the rhAPC treatment group experienced improved 28-day survival. At 28 days, the observed mortality rates were 24.71% for the rhAPC treatment group and 30.83% for

the placebo treatment group (nonstratified p=0.0049). These results represent an absolute risk reduction of 6.12% with reference to mortality, corresponding to a 19.87% reduction in the relative risk of death, a 26.39% reduction in the odds of death, and a 35.85% increase in the odds of survival in the rhAPC treatment group compared with the placebo treatment group.

A statistically significant reduction in 28-day all-cause mortality was observed in patients (*all enrolled patients*) randomly assigned to rhAPC treatment compared with those assigned to placebo treatment (p=0.0027). Relative to the placebo treatment group, the rhAPC treatment group experienced a 20.70% reduction in the relative risk of death, a 27.52% reduction in the odds of death, and a 37.97% increase in the odds of survival.

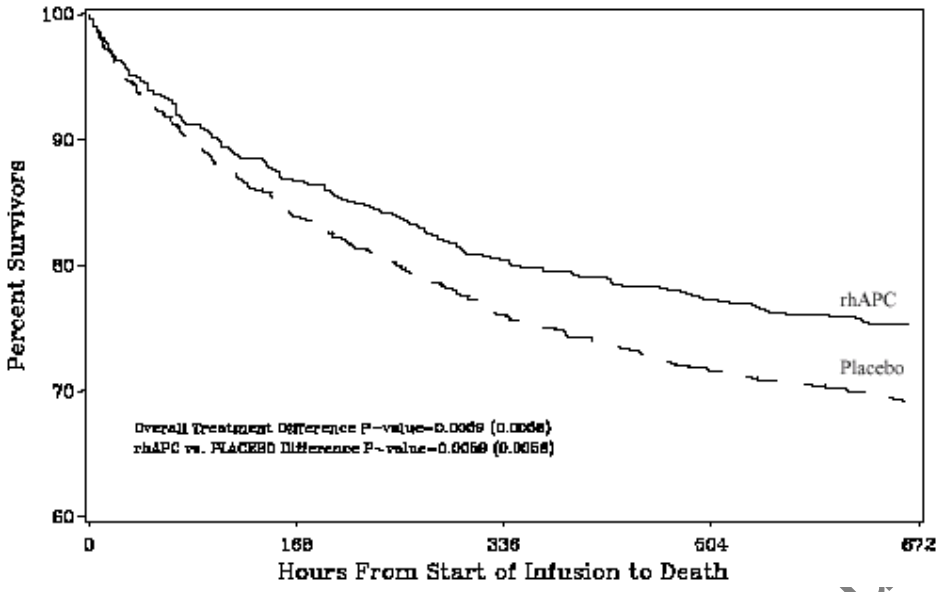
Treatment Group	Deceased	Survived	Total
rhAPC	210 (24.71%)	640 (75.29%)	850 (50.3%)
Placebo	259 (30.83%)	581 (69.17%)	840 (49.7%)
All Patients	469 (27.75%)	1221 (72.25%)	1690 (100%)

The results of the prospectively defined (*stratified*) primary mortality analysis were consistent with the previous findings. A statistically significant reduction in 28-day all-cause mortality was observed for patients receiving rhAPC compared with those receiving placebo (p=0.0054). A 19.43% reduction in the relative risk of death, a 27.59% reduction in the odds of death, and a 38.1% increase in the odds of survival were observed comparing the rhAPC treatment group with the placebo treatment group.

Analysis	p-Value[a]	Relative Risk	Relative Risk 95% CI	Odds Ratio	Odds Ratio 95% CI	Interaction
Primary Analysis – Primary Stratification with Pooling	0.0054	0.8057	0.6950–0.9339	0.7241	0.5735–0.9143	0.2359
Primary Stratification with No Pooling	0.0027	0.8215	0.7088–0.9522	0.7288	0.5755–0.9230	0.0585
Nonstratified	0.0049	0.8013	0.6862–0.9356	0.7361	0.5943–0.9116	—

[a] Two-sided p-value from the Cochran-Mantel-Haenszel test for stratified analyses and Pearson's chi-square test for the nonstratified analysis.

The following figure displays the Kaplan-Meier survival curves for the ITT population by treatment group. A statistically significant increase in survival was observed for the rhAPC treatment group compared with the placebo treatment group (stratified p=0.0056, nonstratified p=0.0059).



There was no consistent treatment effect in subgroups defined by APACHE II disease severity score at baseline. (The APACHE II score is designed to assess the risk of mortality based on acute physiology and chronic health evaluation).
In the subgroup of patients with an APACHE II score >25 at baseline, the mortality was 31% in the Xigris group (128 out of 414) and 44% in the placebo group (176 out of 403). In the subgroup of patients with at least 2 acute organ dysfunctions at baseline, the mortality was 26.5% in the Xigris group (168 out of 634) and 33.9% in the placebo group (216 out of 637). No significant death reduction was observed in the subgroup of patients with less than 2 acute organ dysfunctions at baseline.

No improvement in mortality in first APACHE II quartile was observed and only a marginal one in the second quartile; taken together, for the patients included in the 1st and 2nd APACHE II quartiles no improvement in mortality was apparent. A borderline statistically significant treatment-by-APACHE-II-quartile interaction was observed based on the Breslow-Day test for homogeneity of odds ratios across strata (p=0.0899). The observed interaction was primarily due to the difference in the odds of death observed in the first APACHE II quartile compared with the second, third, and fourth quartiles. In the first APACHE II quartile 15% of the patients died in the rhAPC group in comparison to 12% in the placebo group (RR= 1.2518). The observed relative risk reductions in 28-day all-cause mortality with rhAPC treatment compared with placebo treatment were greatest for patients in the third and fourth APACHE II quartiles (34.28% and 22.20%, respectively).

Based on the efficacy results from the single phase III study, there seems to be a clear clinical benefit of treatment with APC in a subgroup of patients with more severe sepsis, defined as patients belonging to the 3rd and 4th APACHE II quartile.
There is a need for further clinical data in patients with less severe sepsis (e.g. APACHE II score ≤24). The Company will provide further safety and efficacy data in sepsis patients at lower risk of death as soon as they are available.

The selection of appropriate patients for rhAPC treatment based on APACHE II disease severity scores would not be clinically manageable. There was therefore a need to translate the inclusion criteria for the phase 3 study into clinically meaningful terms, such as severe sepsis, organ dysfunction and/or shock in a way that could be acceptable from both a clinical and regulatory perspective. This has been appropriately reflected in the SPC.

The observed relative risk reductions in 28-day all-cause mortality with rhAPC treatment compared with placebo treatment were similar across age classes. (A statistically significant treatment-by-age-class (<75 or ≥75 years) interaction was observed based on the Breslow-Day test for homogeneity of

odds ratios across strata (p=0.0826). The observed relative risk reduction in 28-day all-cause mortality in the rhAPC treatment group compared with the placebo treatment group was greatest for patients ≥75 years (31.55%) compared with patients <75 years (15.26%). However, this pattern was not observed in the age class of <65 or ≥65. The observed relative risk reduction in 28-day all-cause mortality in the rhAPC treatment group compared with the placebo treatment group was 18.52% for patients ≥65 years and 25.67% for patients <65 years).

Patients in the highest quartile of rhAPC plasma concentrations (C_{ss}) have a significantly higher mortality (33.3 % *vs* 21.2 % for the entire population analyzed and *vs* 14.8 % for the first quartile). There is a clear trend for higher mortality in patients with higher plasma rhAPC concentrations (P=0.017). Some elements suggests that patients with the lower rhAPC clearance (fourth quartile) were more likely to be older, have higher baseline APACHE II scores, higher baseline AST and ALT concentrations, more abnormal renal function and longer PT at baseline.

The observed relative risk reduction in 28-day all-cause mortality in the rhAPC treatment group compared with the placebo treatment group was greater for men (21.57%, 95% CI = [0.6380-0.9641]) than for women (17.63%, 95% CI = [0.6512-1.0420]).

A statistically significant treatment-by-Protein-C-activity-class interaction was observed based on the Breslow-Day test (p=0.0403). The observed relative risk reductions in 28-day all-cause mortality with rhAPC treatment compared with placebo treatment were greatest for patients in the ≤40% baseline Protein C activity class (33.96%) and in the >80% Protein C activity class (41.67%). There was no statistically significant treatment-by-subgroup interaction observed based on subgroups defined by baseline Protein C deficiency status (p=0.3338). In addition, a logistic regression analysis of 28-day survival status did not yield a statistically significant treatment-by-baseline-Protein-C-activity-level interaction (p=0.731).

A non-statistically significant increase of 9.78% in the relative risk of death for rhAPC-treated patients (27.08%) compared with placebo-treated patients (24.67%) was observed in the subgroup of patients with a baseline Protein C activity level between 41% and 60% (p=0.5518). In this subgroup, a lower percentage of rhAPC-treated patients had the urinary tract identified as their primary site of infection (7.5% versus 14.5%) or had recent trauma as a solicited historical diagnosis (1.7% versus 4.8%) relative to placebo-treated patients. Among placebo-treated patients in the ITT population, those with urinary tract as the primary site of infection or recent trauma as a historical diagnosis had lower 28-day all-cause mortality than the ITT placebo population as a whole (20.93% versus 30.83%, urinary tract infection; 23.26% versus 30.83%, recent trauma). When Protein C level is analyzed as a continuous variable, no relationship is seen between the relative risk reduction in mortality and baseline Protein C activity level. These findings suggest a treatment benefit associated with rhAPC administration in patients with a clinical diagnosis of severe sepsis irrespective of the baseline Protein C activity level.

An amended protocol was approved and 970 patients were enrolled and received the study medication under this version of the protocol. Patients enrolled under the amended protocol were less likely to have chronic health points as a component of the APACHE II score, and were less likely to have metabolic acidosis as an organ failure. However, the overall APACHE II score remained similar and patients enrolled under the amended protocol had higher median baseline IL-6 levels (565.80 pg/mL versus 388.55 pg/mL), suggesting that there was no resulting reduction in the severity of the acute sepsis episode. These findings suggest that the amended protocol was successful in increasing the percentage of patients who had sepsis as their primary determinant of mortality.

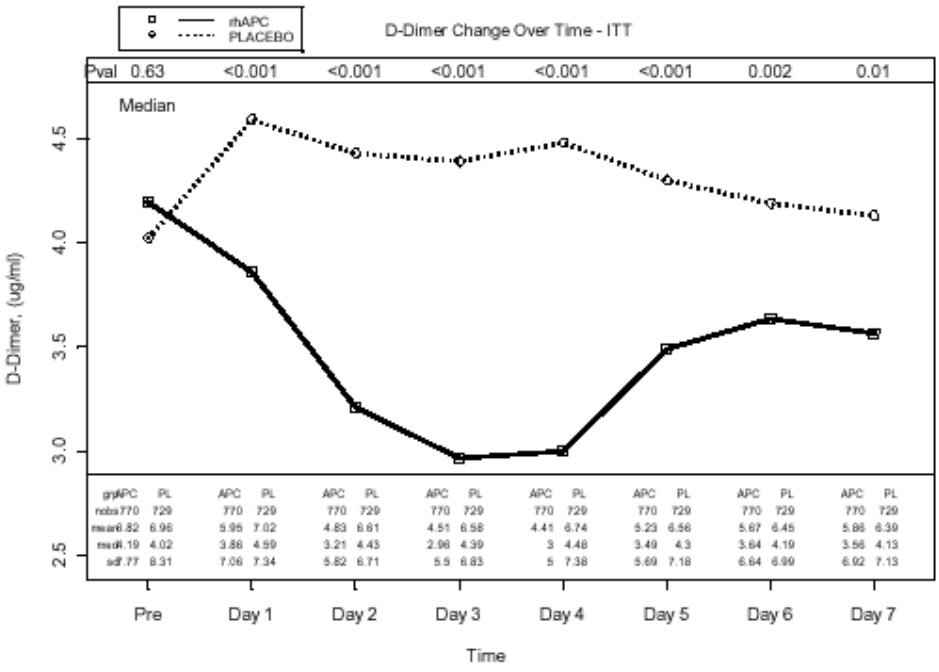
The other main change related to the protocol amendment, to further ensure the integrity of the study blinding, was the introduction of human serum albumin as placebo (apart from investigation centers located in France for regulatory reasons). The concentration used for the placebo infusion was 1 mg/mL (0.1% in normal saline), the resulting daily dose was 100-fold less than the recommended daily therapeutic dose of human serum albumin and is 300- to 600-fold less than the maximum dose.

D-dimer analysis

D-dimer levels were determined at baseline (within 24 hours prior to study drug infusion), daily at days 1 through 7, and at days 14 and 28. D-dimer analyses included only those patients with available baseline and post-baseline data.

D-dimer levels were statistically significantly lower for rhAPC-treated patients compared with placebo-treated patients on Study Day 1 ($p<0.001$) and on Study Days 2 through 7, (all $p\leq0.014$). The more rapid decrease in D-dimer levels observed for rhAPC-treated patients is indicative of the antithrombotic pharmacodynamic properties of the molecule. D-dimer levels rose following the end of study drug infusion in the rhAPC treatment group, but remained below baseline levels. This rise in D-dimer levels may reflect ongoing thrombin generation due to sepsis or the restoration of normal fibrinolysis, as D-dimer is also a product of fibrinolysis.

Median D-dimer levels on Study Days 1 through 7 for the ITT population in Study F1K-MC-EVAD.



Other endpoints

Various other biomarkers were measured to assess rhAPC effect on thrombin generation. The clinical data support the *in vitro/ex vivo* data that rhAPC does not function purely as an antithrombotic. RhAPC may also have anti-inflammatory properties evidenced by a more rapid fall in interleukine-6. The relevance of this effect, however, when assessing the anti-inflammatory properties of drotrecogin alfa (activated) is unclear. A greater fall in PAI-1 levels was recorded in the rhAPC treatment group. The greater increases in plasminogen levels recorded in the rhAPC treatment group cannot be regarded as a qualified marker for demonstration of profibrinolytic effects of rhAPC.

Concomitant medications

A statistically significant treatment-by-concomitant heparin-exposure interaction was observed based on the Breslow-Day test for homogeneity of odds ratios across strata (p=0.026) in a post-baseline analysis (see table). The observed relative risk reduction in 28-day all-cause mortality for the rhAPC treatment group compared with the placebo group was greatest for patients not exposed to heparin (38.91%) than for patients who were exposed to heparin (11.31%).

Summary of 28-Day Mortality by Heparin Exposure
Study F1K-MC-EVAD

	Drotrecogin Alfa (Activated)		Placebo		
	No. of Patients (%)	Died n (%)	No. of Patients (%)	Died n (%)	Interaction p-Value
Concomitant Prophylactic Heparin Exposure[a]					
With Heparin	634 (74.6)	158 (24.9)	637 (75.8)	179 (28.1)	0.026
No Heparin	216 (25.4)	52 (24.1)	203 (24.2)	80 (39.4)	
Baseline Prophylactic Heparin Exposure[b]					
With Heparin	532 (62.6)	138 (25.9)	559 (66.5)	170 (30.4)	0.298
No Heparin	318 (37.4)	72 (22.6)	281 (33.5)	89 (31.7)	

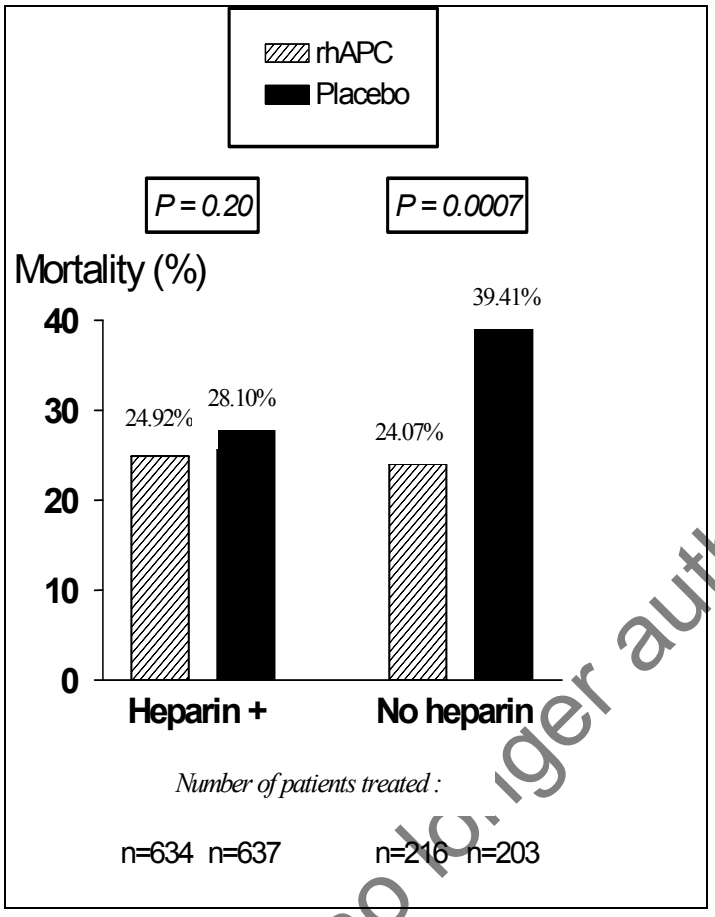
Abbreviations: No. = number.

[a] Includes all patients who received any heparin during the study drug infusion or the next calendar day after the completion of the infusion.

[b] Includes all patients who were receiving heparin at baseline (the day before or the day of the start of study drug infusion).

Source: Table EVAD.11.89, Clinical Study Report F1K-MC-EVAD; SAS Output.

The analysis indicates a significant difference in mortality between patients having received heparin as concomitant therapy as compared to those who did not. In the rhAPC-treated-group there was no difference in mortality rate between patients who received heparin (24.92%) and those who did not (24.07%). In the placebo-treated-group, however, there was a statistically significant difference in mortality rate between those who had received heparin (28.10%) and those who had not (39.41%). The highest mortality rate was reported in the placebo-treated-group with no heparin. In this post-baseline analysis the mortality rates in the three other groups (rhAPC with or without heparin, and placebo with heparin) were similar.



The Phase 3 trial was designed to have adequate power for the comparison of all rhAPC patients to all placebo patients. However, post hoc analysis comparing the mortality rates in 4 subgroups defined by exposure to prophylactic dose heparin either at baseline and/or during study drug infusion were performed (see table below). Although based on subgroups modified post randomisation and therefore subject to bias, these analyses suggest that the efficacy of drotrecogin alfa activated was most evident in the subgroup of patients who were never exposed to heparin (see D vs. D'). This subgroup represents only 25 % of the whole population. It is also noted that within the subgroup of patients being treated with prophylactic dose heparin at baseline (65 % of the whole population) and who were randomised to rhAPC vs. placebo, no statistically significant effect of rhAPC was demonstrated (within subgroup P=0.106 if one considers all patients with heparin at baseline [N=1091], and P=0.172 if one considers the subgroup of patients receiving heparin both pre- and post-baseline [N=1056]).

A difference in mortality rate between the three groups of patients who were exposed to heparin and/or rhAPC (i.e., rhAPC with concomitant heparin, rhAPC without concomitant heparin, and placebo with concomitant heparin) could not be demonstrated by a chi-square test (P=0.328).

Mortality by prophylactic dose heparin exposure at baseline and or during study medication infusion.

Group	Baseline Heparin	Post-Baseline Heparin during study drug infusion period	28-Day Mortality Results
A. rhAPC	Yes	Yes	132/515 (25.6%)
B. rhAPC	Yes	No	6/17 (35.3%)
C. rhAPC	No	Yes	26/119 (21.9%)
D. rhAPC	No	No	46/199 (23.1%)
A'. placebo	Yes	Yes	159/541 (29.4%)
B'. placebo	Yes	No	11/18 (61.1%)
C'. placebo	No	Yes	20/96 (20.8%)
D'. placebo	No	No	69/185 (37.3%)

Statistical Comparison	Observed Relative Risk of Death with 95% confidence interval rhAPC vs. Placebo	Chi-square test p-value
A versus A'	0.87 (0.72 – 1.06)	0.172
B versus B'	0.58 (0.28 – 1.21)	0.127
C versus C'	1.05 (0.63 – 1.76)	0.857
D versus D'	0.62 (0.45 – 0.85)	0.002
A, B, C versus A', B', C'	0.87 (0.73 – 1.04)	0.121
A, B versus A', B'		0.106

To summarise, two-thirds of patients in the phase 3 trial received prophylactic doses of unfractionated or low molecular weight heparin. There was no observed increase in the risk of bleeding events reported as serious adverse events in drotrecogin alfa (activated) patients receiving heparin. The effects of prophylactic low dose heparin and other coagulation-active medications on the efficacy of drotrecogin alfa (activated) have not been evaluated in a randomized controlled clinical trial.
However, some evidence from recent *in vitro* studies seems to suggest that an interaction between heparin and APC might be biologically plausible. Furthermore, the findings of the retrospective post-hoc analysis of the phase 3 study seem to indicate that concomitant treatment with low dose heparin could potentially result in a decreased effect of APC. Therefore, it cannot totally be excluded that the co-administration of low dose heparin with drotrecogin alfa (activated) could potentially reduce the efficacy of the drotrecogin alfa (activated). Further drug interaction studies with heparin (as well as other coagulation active medications) are needed, since it is likely that septic patients might receive treatment with one of these drugs due to underlying disease/condition.
The applicant has committed to undertake a further specific clinical study to investigate the potential interaction of heparin and APC. Furthermore, the applicant will seek Scientific Advice prior to initiation of such a study.

No data on treatment with drotrecogin alfa activated and other coagulation active medications than heparin are available since patients receiving these medications were not enrolled in the study.
Caution should therefore be employed when Xigris is used with other drugs that affect haemostasis including Protein C, thrombolytics (e.g. streptokinase, tPA, rPA and urokinase), oral anticoagulants (e.g. warfarin), hirudins, antithrombin, aspirin and other anti-platelets agents, e.g. non-steroidal anti-inflammatory drugs, ticlopidine and clopidogrel, glycoprotein IIb/IIIa antagonists (such as abciximab, eptifibatide, tirofiban) and prostacyclins such as iloprost. This has been appropriately reflected in the SPC.

Clinical studies in special populations

A study in paediatric patients with severe sepsis has been conducted (Study F1K-MC-EVAO). This study was an open-label study that assessed the safety, pharmacokinetics, and pharmacodynamics of drotrecogin alfa activated administration in paediatric patients from newborn to <18 years of age. The objectives of this study were to provide evidence that the course of severe sepsis is similar in paediatric patients and adult patients and that the beneficial effect and safety profile of drotrecogin alfa activated administration is similar in paediatric as in adult patients. Comparability of the disease state between the two patient populations was assessed by clinical evidence, clinical history, and baseline measurements of coagulation markers (D-dimer, Protein C, and antithrombin). Comparability of the effect of rhAPC administration in the two patient populations was further assessed by observed changes in plasma D-dimer concentrations.

The available data obtained from this study suggest that baseline data describing the disease process for paediatric patients with severe sepsis in Study F1K-MC- EVAO are similar to baseline data describing the disease process observed for adult patients in Study F1K-MC-EVAD.

These data suggest that both the pharmacodynamic properties and the safety profile of rhAPC were similar in paediatric and adult patients with severe sepsis. The experience with drotrecogin alfa activated in children under the age of 18 is limited; the efficacy and safety of Xigris have not been established in this age group; therefore no dosage recommendation can be made. This has been adequately reflected in the SPC. The Company has committed to performing a further clinical trial to gather more data in paediatric patients including purpura fulminans.

Clinical safety

Drotrecogin alfa (activated) exhibits antithrombotic and antifibrinolytic properties. Due to these pharmacological properties, an increased risk of bleeding would be expected in patients receiving the medication.

- Patient exposure

Eight phase I clinical studies were conducted and evaluated the safety and pharmacokinetic properties of recombinant human Activated Protein C (rhAPC) in healthy subjects (seven studies) and in patients with end-stage renal disease (one study). A total of 112 unique subjects were exposed to rhAPC during these studies. In one phase Ib study (F1K-MC-EVAB) in heterozygous Protein-C deficient patients, 6 patients received rhAPC. In the two-stage phase II (duration and dose ranging study) and phase III studies (24 mcg/kg/h during 96 hours), 940 patients were exposed to rhAPC, in comparison to 881 to placebo.

The overall rate of serious adverse events (SAE) related to drotrecogin alfa (activated) (as reported by the Applicant) ranges from 2.1% in the 850 patients participating in Study F1K-MC-EVAD, to 3.3% in 1158 patients in the open-label trials F1K-MC-EVBE, F1K-MC-EVBF, and F1K-MC-EVBG to 5.4% in 258 patients in the compassionate use trial, F1K-MC-EVBC, and 3.5% in the patients receiving treatment outside of clinical trials.

- Adverse events related to general disorders and administration site conditions: Deaths

There were no deaths among the subjects in the phase 1/1b studies. In the phase 2/3 studies, the majority of deaths in both treatment groups were due to either refractory septic shock or sepsis-induced multi-system organ failure. A total of 6 patients in the rhAPC treatment group and 2 patients in the placebo treatment group experienced haemorrhage as a potential cause of death. Four of these haemorrhagic events were assessed by the investigators as being possibly related to study medication (all four in the rhAPC treatment group). In each case, the patient was predisposed to bleeding as a result of a severe underlying coagulopathy or as a result of trauma to a major artery.

There were 509 (rhAPC: 236, placebo: 273) deaths reported in the completed Phase 2/3 studies. Among the 509 deaths, 6 were reported to be study-medication related by the investigator (rhAPC: 5, placebo: 1). Four of the rhAPC-treated related deaths were associated with bleeding serious reactions: 2 patients experienced fatal cerebral haemorrhage, 1 patient experienced fatal pulmonary haemorrhage, and 1 patient experienced a fatal haemorrhage from an aortic disruption. A fifth rhAPC-treated patient experienced cerebral oedema and severe hypoxia. The patient in the placebo treatment group developed cerebral infarcts and died. These deaths are all included in the overall mortality analyses.

The 28-day all-cause mortality of patients with severe sepsis was the primary endpoint in the Pivotal study (Pivotal Phase 3 EVAD study). A statistically significant treatment-by-surgical status on the incidence of death was observed (p=0.0538). For emergency post-operative patients, increased 28-day mortality was observed for rhAPC-treated patients compared with placebo-treated patients. However, the observed difference in mortality within the emergency post-operative patient stratum between the treatment groups was not statistically significant. Analyses of treatment-emergent bleeding events by surgical status that occurred during the study drug infusion period indicated no significant interaction between treatment group and surgical status for the odds of experiencing a treatment-emergent bleeding event.

As previously discussed a statistically significant interaction with heparin exposure has been observed. The observed relative risk reduction of mortality for the rhAPC treatment group compared to the placebo treatment group was greatest for patients not exposed to heparin (38.91%) than for patients who were exposed to heparin (11.31%). The observed 28-day all-cause mortality for rhAPC-treated patients was similar for patients who were exposed or not exposed to heparin (24.92% versus 24.07%).

The incidence of death (28-day all-cause mortality) was also examined by looking at the relationship between maximum activated partial thromboplastin time and prothrombin time and minimum platelet count during study days 1 through 5 (a time period similar to the study drug infusion period for the majority of patients). There was an increased incidence of death as maximum activated partial thromboplastin time and maximum prothrombin increased and minimum platelet count decreased within both the rhAPC and placebo treatment groups. There was a decreased risk of dying for the patients enrolled in the rhAPC treatment group compared to placebo for all three categories examined. The risk reduction remained fairly constant with no statistically significant interaction between these parameters and odds of death. A similar pattern was seen when death was analysed by baseline coagulation profile.

- Adverse events related to general disorders and administration site conditions: Haemorrhage NOS – Bleeding

APC has the potential to increase the risk of bleeding.

No cases of serious bleeding events were reported in the completed Phase 1/1B studies. In the phase 1 studies, adverse events with a frequency of ≥ 5% included headache (30.9%), ecchymosis (23.0%), and pain (5.8%).

The percentage of patients experiencing at least one bleeding event in the Phase 3 clinical trial involving 850 drotrecogin alfa (activated)-treated and 840 placebo-treated patients was 24.9% and 17.7%, respectively. In both treatment groups, the majority of bleeding events were ecchymosis or gastrointestinal tract bleeding.

In the completed Phase 2/3 studies, the rhAPC treatment group had a statistically significantly greater percentage of patients who experienced at least one bleeding event considered as being serious during the study drug infusion period (2.4% versus 0.9%) and during the 28-day study period (3.6% versus 2.0%) compared with the placebo treatment group. The absolute difference in the incidence of serious bleeding events between the two treatment groups was 1.5% during the study drug infusion period and

1.6% during the 28-day study period. Serious bleeding events were defined as any intracranial haemorrhage, any life-threatening bleed or any bleeding event requiring the administration of ≥ 3 units of packed red blood cells per day for 2 consecutive days, or any bleeding event assessed as serious by the investigator. Increased bleeding with rhAPC was seen only during the study drug infusion, which is consistent with its short half-life. Serious bleeding events were almost always associated with vessel trauma, tissue trauma (either accidental or iatrogenic), or ulceration of the gastrointestinal tract.

In the pivotal phase III study, the percentage of patients who had at least one serious adverse event was similar in the two groups and the incidence of serious bleeding episodes was higher in the drotrecogin alfa (activated) group than in the placebo group (3.5 percent vs. 2.0 percent) (p=0.06). The difference in the incidence of serious bleeding was only observed during the study drug infusion period. In both the drotrecogin alfa (activated) and the placebo group serious bleeding occurred primarily in patients with an identifiable predisposition to bleeding such as gastrointestinal ulceration, an activated partial thromboplastin time of more than 120 seconds, a prolonged prothrombin time (corresponding to an international normalized ratio of more than 3.0), a platelet count that decreased to less than 30,000 per cubic millimetre and remained at that level despite standard therapy, traumatic injury of a blood vessel, or traumatic injury of a high vascular organ. After adjustment for the duration of survival, blood-transfusions requirements were similar in the two groups (p=0.90).

A statistically significantly greater percentage of rhAPC-treated patients experienced gastrointestinal haemorrhage, ecchymosis, injection site haemorrhage, melaena, and rectal haemorrhage during the study drug infusion period, whereas more placebo-treated patients experienced haemoptysis. In both treatment groups, the majority of the bleeding events were mild (10.1% versus 7.4%) or moderate (5.1% versus 1.8%) in severity. Severe bleeding events occurred less frequently but were similarly more common in the rhAPC treatment group (2.7% versus 1.2%).

There was no interaction between surgical status and incidence of bleeding events. However, the bleeding event rate in the placebo-treated surgical patients appeared lower than in the non-surgical patients. This may have reflected the exclusion criteria of surgery within 12 hours, which may have produced populations with differing bleeding risks. The numbers of surgical patients with a bleeding event was also small, making it difficult to draw firm conclusions from these data.

In the phase 3 study, 2 cases of intracranial haemorrhage (ICH) occurred during the infusion period for drotrecogin alfa (activated)-treated patients and no cases were reported in the placebo-treated patients. In non-placebo controlled trials, ICH has been reported in patients receiving drotrecogin alfa (activated) with an incidence of approximately 0.9% during the infusion period. The estimation of spontaneously reported ICHs is found to be similar (0.2%). The risk of ICH may increase with severe coagulopathy and severe thrombocytopenia. The risk of ICH was higher in patients treated with Xigris in compassionate studies than in non-compassionate studies. The difference might be explained by different risk profiles of the study populations at entry.

The incidence of serious bleeding in the ongoing open studies is 3.9% during infusion period and 6.6% during study period. The spontaneous reporting rate has been 1.1%. The assessment of the non-bleeding events may be confounded by underlying disease. The events might be explained by complications of sepsis. It has to be noted that it is difficult to assess the true incidence of an event using spontaneous data, due to the unknown effect of underreporting.

It is feasible to identify the patients at risk of bleeding prior to treatment and exclude them from therapy on the bases of the exclusion criteria related to an increased risk of bleeding, (as defined by the study protocol) that are included as contraindications in section 4.3 of the SPC.
Nevertheless, there is a need for a thorough analysis of currently available data, close monitoring of reports and serious adverse events during the post-marketing phase as well as data from further studies. The applicant has committed to address all serious bleeding events every 6 months by providing a detailed section on bleeding in the PSURs. The first PSUR will be provided within 60 days of 21st November 2002, which is one year after the International Birth Date.

- Other safety issues

For the combined studies EVAA and EVAD, there was no statistically significant difference between the rhAPC treatment group relative to the placebo treatment group in the percentage of patients who experienced at least one serious adverse event during the study drug infusion period (7.3% versus 6.8%) and during the 28-day study period (13.2% versus 12.9%). There also were no serious adverse events (other than the bleeding event haemorrhage), individually or grouped by body system, for which differences between treatment groups were statistically significant during the study drug infusion period and during the 28-day study period.

The percentage of patients experiencing at least one adverse reaction was not statistically significantly different between the rhAPC treatment group and the placebo treatment group during the study drug infusion period (67.6% and 64.4%, respectively) and during the 28-day study period (80.2% and 77.2%, respectively).

Mild headache was the most common adverse event reported in subjects in Phase 1 studies. The incidence of headache was dose but not dose duration dependent. The aetiology of headache is unclear. Ecchymosis was also frequently reported. Ecchymosis was reported to occur most often at the site of venipuncture. Additionally, there was a higher than expected occurrence of occult blood in the urine in healthy subjects. Both of these findings are very likely to be the result of the antithrombotic and profibrinolytic properties of rhAPC.

- Laboratory findings

Clinical laboratory evaluations of serum electrolytes; chemistry and haematology panels, urinalysis; and platelet function were performed in Phase 1 studies. Administration of drotrecogin alfa (activated) in subjects did not appear to be associated with abnormalities in either chemistry or haematology laboratory parameters.

In both Studies F1K-MC-EVAA and F1K-MC-EVAD extensive analyses of the central chemistry and haematology data were conducted. Based on the assessments of the central laboratory data in each study, there were no identifiable safety concerns associated with rhAPC administration.

- Immunogenicity

No antibody response was detected in any of the subjects in Phase 1/1B studies (n=104) exposed to drotrecogin alfa (activated), even upon multiple re-administrations. A majority (87%) of the subjects participating in the Phase 1/1B studies were exposed to rhAPC more than once, and 18% of these subjects were exposed to rhAPC four to six times.

In patients with severe sepsis exposed to rhAPC, anti-APC antibody formation was uncommon (2/370 patients; 0.54%), was low in titre, and was unrelated to the specific bulk drug substance administered (BDS2 versus BDS2+). These anti-activated protein C antibodies were not able to neutralise the effect of plasma-derived human derived activated protein C or rhAPC on the activated partial thromboplastin time assay. Both patients in whom anti-activated protein C antibodies were detected survived to Study Day 28. Antibody formation in one patient was transient and not associated with any clinical adverse reaction. The other patient did experience superficial and deep venous thromboses that were not considered as serious by the investigator. No follow-up beyond the study at day 28 is available for this patient. In the Phase 2 and Phase 3 studies, the incidence rate of thrombosis was higher in placebo-treated patients than in rhAPC-treated patients. To date, there is no re-administration experience of rhAPC in patients with severe sepsis.
The possibility of allergic reactions to constituents of the preparation cannot be completely excluded in certain predisposed patients. If allergic or anaphylactic reactions occur, treatment should be discontinued immediately and appropriate therapy initiated. Xigris has not been readministered to patients with severe sepsis. If Xigris is readministered to patients, caution should be employed. No anti-activated Protein C antibody formation was detected in healthy subjects, even after repeat administration. This has been appropriately reflected in the SPC.

Discussion on Clinical aspects

Discussion on clinical efficacy

Xigris was studied in one Phase 3 international, multi-centre, randomized, double-blind, placebo-controlled trial (PROWESS) in 1690 patients with severe sepsis. Severe sepsis is defined as sepsis associated with acute organ dysfunction. Patients meeting the clinical diagnosis of severe sepsis had a) known or suspected infection, b) clinical evidence of systemic response to infection including fever or hypothermia, leucopenia or leucocytosis, tachycardia and tachypnoea, and c) acute organ dysfunction. Organ dysfunction was defined as shock, hypotension or the need for vasopressor support despite adequate fluid resuscitation, relative hypoxemia (ratio of partial pressure of oxygen in arterial blood in mmHg to the percentage of oxygen in the inspired air expressed as a decimal (PaO_2/FiO_2 ratio) < 250), oliguria despite adequate fluid resuscitation, marked reduction in blood platelet counts, and/or elevated lactic acid concentrations.

Exclusion criteria encompassed patients at high risk of bleeding (see Sections 4.3 and 4.4 of the SPC), patients who were not expected to survive for 28 days due to a pre-existing, non-sepsis related medical condition, HIV positive patients whose most recent CD_4 count was ≤ 50/mm^3, patients on chronic dialysis, and patients who had undergone bone marrow, lung, liver, pancreas or small bowel transplantation, and patients with acute clinical pancreatitis without a proven source of infection.
The inclusion and exclusion criteria of this phase 3 study are considered appropriate to define the target population for APC treatment and has been reflected in the SPC.

Patients were given a 96-hour constant rate infusion of Xigris at 24 µg/kg/hr (n=850) or placebo (n=840). Xigris was added to best standard care. Best standard care includes adequate antibiotics, source control and supportive treatment (fluids, inotropes, vasopressors and support of failing organs, as required).

Patients treated with Xigris experienced improved 28-day survival compared to those treated with placebo. At 28 days, the overall mortality rates were 24.7% for the Xigris-treated group and 30.8% for the placebo-treated group (p=0.005).

In patients with severe sepsis, the plasma clearance of rhAPC was significantly decreased by renal impairment and hepatic dysfunction, but the magnitude of the differences in clearance (<30%) does not warrant any dosage adjustment in patients with renal disease. Understandably, there was no specific study in patients with hepatic disease, since the risk of bleeding would be unacceptably high.
In addition, no dose adjustments are required in adult patients with severe sepsis with regard to age, gender, hepatic function (as measured by transaminase levels). The pharmacokinetics of drotrecogin alfa (activated) have not been studied in patients with severe sepsis and preexisting endstage renal disease and chronic hepatic disease. This is appropriately reflected in the SPC.

Significant absolute death reduction was limited to the subgroup of patients with greater disease severity i.e. baseline APACHE II score >25 or at least 2 acute organ dysfunctions at baseline. (The APACHE II score is designed to assess the risk of mortality based on acute physiology and chronic health evaluation). In the subgroup of patients with an APACHE II score >25 at baseline, the mortality was 31% in the Xigris group (128 out of 414) and 44% in the placebo group (176 out of 403). No death reduction was observed in the subgroup of patients with lower disease severity. In the subgroup of patients with at least 2 acute organ dysfunctions at baseline, the mortality was 26.5% in the Xigris group (168 out of 634) and 33.9% in the placebo group (216 out of 637). No significant death reduction was observed in the subgroup of patients with less than 2 acute organ dysfunctions at baseline.

There is a need for further clinical data in patients with less severe sepsis (e.g. APACHE II score ≤24). The Company will provide further safety and efficacy data in sepsis patients at lower risk of death as soon as they are available.

Approximately 2/3 of the patients received prophylactic low dose heparin during the course of study. The mortality rate in patients receiving Xigris and concomitant prophylactic low dose heparin was 24.9% and the mortality rate in patients receiving placebo and concomitant prophylactic low dose heparin was 28.1% (p=0.20). There is uncertainty if heparin could interfere with the activity of Xigris. The effect of low dose heparin on the efficacy of Xigris has not been evaluated in specific randomised controlled clinical trials.
Further drug interaction studies with heparin (as well as other coagulation active medications) are needed, since it is likely that septic patients might receive treatment with one of these drugs due to underlying disease/condition.
The applicant has committed to undertake a further specific clinical study to investigate the potential interaction of heparin and APC. Furthermore, the applicant will seek Scientific Advice prior to initiation of such a study.
No data on treatment with drotrecogin alfa (activated) and other coagulation active medications than heparin are available since patients receiving these medications were not enrolled in the study.
Caution should therefore be employed when Xigris is used with other drugs that affect haemostasis including Protein C, thrombolytics (e.g. streptokinase, tPA, rPA and urokinase), oral anticoagulants (e.g. warfarin), hirudins, antithrombin, aspirin and other anti platelets agents, e.g. non-steroidal anti-inflammatory drugs, ticlopidine and clopidogrel, glycoprotein IIb/IIIa antagonists (such as abciximab, eptifibatide, tirofiban) and prostacyclins such as iloprost. This has been appropriately reflected in the SPC.

The results of the clinical programme suggest that both the pharmacodynamic properties and the safety profile of rhAPC were similar in adult and paediatric patients with severe sepsis. The experience with drotrecogin alfa activated in children under the age of 18 is limited; the efficacy and safety of Xigris have not been established in this age group; therefore no dosage recommendation can be made. This has been adequately reflected in the SPC. The Company has committed to performing a further clinical trial to gather more data in paediatric patients including purpura fulminans.

Discussion on clinical safety

Xigris has the potential to increase the risk of bleeding. The percentage of patients experiencing at least one bleeding event in the Phase 3 clinical trial involving 850 drotrecogin alfa (activated)-treated and 840 placebo-treated patients was 24.9% and 17.7%, respectively. In both treatment groups, the majority of bleeding events were ecchymosis or gastrointestinal tract bleeding.
In the Phase 3 clinical trial, serious bleeding events occurred in 3.5% and 2.0% in drotrecogin alfa (activated)-treated and placebo-treated patients, respectively. Serious bleeding events were defined as any intracranial haemorrhage, any life-threatening bleed or any bleeding event requiring the administration of ≥ 3 units of packed red blood cells per day for 2 consecutive days, or any bleeding event assessed as serious by the investigator. The difference in the incidence of serious bleeding events between the two treatment groups occurred primarily during study drug administration.

In the phase 3 study, 2 cases of intracranial haemorrhage (ICH) occurred during the infusion period for drotrecogin alfa (activated)-treated patients and no cases were reported in the placebo-treated patients. In non-placebo controlled trials, ICH has been reported in patients receiving drotrecogin alfa (activated) with an incidence of approximately 0.9% during the infusion period. The risk of ICH may increase with severe coagulopathy and severe thrombocytopenia (see sections 4.3 and 4.4 of the SPC).
The risk of ICH was higher in patients treated with Xigris in compassionate studies than in non-compassionate studies. The difference might be explained by different risk profiles of the study populations at entry.
The incidence of all serious bleeding events in open clinical trials (during study period) is 6.6%.

It is feasible to identify the patients at risk of bleeding prior to treatment and exclude them from therapy on the bases of the exclusion criteria related to an increased risk of bleeding, (as defined by the study protocol) that are included as contraindications in section 4.3 of the SPC. Special attention has also been given to the SPC text under 4.4 Special warnings and special precautions for use.

Nevertheless, there is a need for a thorough analysis of currently available data, close monitoring of reports and serious adverse events during the post-marketing phase as well as data from further studies. Further data on bleeding should be obtained in order to evaluate the incidence if severe/fatal bleeding episodes (intracranial bleedings), incidence of severe/ fatal bleeding episodes overall and the identification of specific subgroups at higher risk of severe bleeding. The applicant has committed to address all serious bleeding events every 6 months by providing a detailed section on bleeding in the PSURs.

The possibility of allergic reactions to constituents of the preparation cannot be completely excluded in certain predisposed patients. If allergic or anaphylactic reactions occur, treatment should be discontinued immediately and appropriate therapy initiated. Xigris has not been readministered to patients with severe sepsis. If Xigris is readministered to patients, caution should be employed. No anti-activated Protein C antibody formation was detected in healthy subjects, even after repeat administration. This has been appropriately reflected in the SPC.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

Xigris is supplied as powder for solution for infusion in two presentations : 20 mg and 5 mg. The active substance, recombinant human Activated Protein C (rhAPC), is obtained by DNA recombinant technology. An inactive zymogen (rhPC), produced by a well characterised primary human cell line (HEK293), is subsequently activated by addition of bovine thrombin, followed by several purification steps before storage or formulation of the drug product.

Issues which remained unsolved at the time of the December 2001 CPMP meeting have all been addressed properly in the last responses of the applicant:

An appropriate potency (APTT) assay has been developed and validated in accordance with Ph. Eur requirements;
All available drug substance and drug product batches have been retested with this new assay, and the submitted results provide assurance on product quality and production consistency, also with regard to the filling strategy proposed by the company. The former has been further confirmed by pre-authorisation sample testing;
In use stability has been checked for commonly available commercial EU sources of infusion sets, and those compatible with the product are mentioned in the SPC.
Additional measures have been taken to ensure the absence of residual bovine thrombin impurities.
The requested information regarding product identity specifications, including justification of specification limits (RP-HPLC, Peptide mapping), has been provided.

In summary, as the application stands now, the chemical, pharmaceutical and biological documentation is of an acceptable quality, and provides a sound basis for the production of a well controlled, consistent product, provided the applicant agrees to the list of commitments issued by the CPMP.

Preclinical pharmacology and toxicology

Overall, pharmacokinetic and pharmacodynamic studies provided adequate evidence in support of the safety and efficacy of drotrecogin alfa (activated). Consistent antithrombotic and fibrinolytic effects associated with the known pharmacology of APC were observed and include, beside the expected prolongation of APTT, decreases in haemoglobin, erythrocytes, and haematocrit, and increases in reticulocyte count and PT. In addition, in vitro studies suggest that APC might exert a direct anti-inflammatory effect, the clinical relevance of which, however, remains unclear.
As activated protein C is a potent antithrombotic, bleeding may be a potential pharmacological toxicity and this was confirmed by the pharmacology and toxicology studies performed. Beyond this, no direct vasoactive effects of rhAPC were demonstrated.

Pharmacodynamic interaction studies revealed an additive effect of unfractionated heparin and rhAPC on the prolongation of *in vitro* activated partial thromboplastin clotting time, which was shown to be almost entirely mediated by reduced antithrombin III levels. It would be very difficult to adequately address the question of potential additive effects of heparin and APC in animal models of sepsis. This issue is therefore discussed further in the clinical part of this report.
Limited animal studies were performed to elucidate any effects of Xigris on pregnancy, embryonal/foetal development, parturition and postnatal development and the potential risk for humans is considered unknown. APC should therefore not be used during pregnancy unless clearly necessary. Furthermore, it is not known whether the product is excreted in human milk or if there is a potential effect on the nursed infant. Therefore, the patient should not breast feed whilst treated with rhAPC. This has been adequately reflected in the SPC.

Efficacy

The results from clinical studies support the use of Xigris in the approved indication *'treatment of adult patients with severe sepsis with multiple organ failure when added to best standard care.'*

In the present state of scientific knowledge, comprehensive information on the safety and efficacy of the medicinal product cannot be provided by the applicant. In order to collect additional data, the applicant has committed to complete a programme of clinical studies post-authorisation, the results of which shall form the basis of an annual re-assessment of the benefit/risk profile.

There is a need for further drug interaction studies with heparin , since it is likely that septic patients might receive treatment with one of these drugs due to underlying disease/condition. The applicant has committed to undertake a specific clinical study to investigate the potential interaction of heparin and APC. Furthermore, the applicant will seek Scientific Advice prior to initiation of such a study. No data on treatment with drotrecogin alfa (activated) and other coagulation active medications than heparin are available since patients receiving these medications were not enrolled in the study, which has been appropriately reflected in the SPC.
In addition, further clinical data in patients with less severe sepsis (e.g. APACHE II score ≤24) are needed. The Company will provide further safety and efficacy data in sepsis patients at lower risk of death as soon as they are available.
The experience with drotrecogin alfa activated in children under the age of 18 is limited; the efficacy and safety of Xigris have not been established in this age group; therefore no dosage recommendation can be made. This has been adequately reflected in the SPC. The Company has committed to perform a further clinical trial to gather more data in paediatric patients including purpura fulminans.

Safety

Safety data show an acceptable adverse event profile and good tolerability to Xigris. However, Xigris has the potential to increase the risk of bleeding which is reflected in the results from the clinical programme.
It is considered feasible to identify the patients at risk of bleeding prior to treatment and exclude them from therapy on the bases of the exclusion criteria related to an increased risk of bleeding, (as defined by the study protocol) that are included as contraindications in section 4.3 of the SPC. Special attention has also been given to the SPC text under 4.4 Special warnings and special precautions for use.
In the present state of scientific knowledge, comprehensive information on the safety and efficacy of the medicinal product cannot be provided by the applicant. In order to collect additional data, the applicant has committed to complete a programme of clinical studies post-authorisation, the results of which shall form the basis of an annual re-assessment of the benefit/risk profile.
There is a need for a thorough analysis of currently available data on bleeding, close monitoring of reports and serious adverse events during the post-marketing phase as well as data from further studies. Further data on bleeding should be obtained in order to evaluate the incidence if severe/fatal bleeding episodes (intracranial bleedings), incidence of severe/ fatal bleeding episodes overall and the identification of specific subgroups at higher risk of severe bleeding. The applicant has committed to

address all serious bleeding events every 6 months by providing a detailed section on bleeding in the PSURs.

Benefit/risk assessment

Following the assessment of the supplementary documentation provided by the applicant, it was concluded that further data was needed to support the quality, safety and efficacy of the product. Although the majority of these questions could be addressed by the applicant as post-authorisation commitments, a number of key issues were identified that needed further discussion at the CPMP's Biotechnology Working Party as well as at an *ad hoc* Expert meeting on clinical aspects of Xigris and at an Oral explanation by the applicant before the CPMP.

The conclusions from the Experts were:

1) The inclusion / exclusion criteria of the single phase III study are appropriate to define the target population for treatment with Xigris; exclusion criteria should clearly be reflected in the appropriate sections of the SPC;
2) Based on the efficacy results from the single phase III study, there seems to be a clear clinical benefit of treatment with Xigris in a subgroup of patients with more severe sepsis, but it was also emphasized that a selection of appropriate patients for Xigris treatment based on APPACHE II disease severity scores, would not be clinically manageable. The inclusion criteria for the phase 3 study should therefore be translated into clinically meaningful terms, such as severe sepsis, organ dysfunction and/or shock in a way that could be acceptable from both a clinical and regulatory perspective;
3) An effect on the efficacy of APC as a result of a possible interaction between low dose heparin and APC cannot be excluded at present; further pre-clinical and/or clinical studies are needed to elucidate the potential interaction of heparin and APC; the currently available data on the concomitant use of heparin should be appropriately reflected as a separate paragraph in section 5.1 'Pharmacodynamic properties' of the SPC;
4) It is feasible to identify the patients at risk of bleeding prior to treatment and exclude them from therapy on the bases of the exclusion criteria related to an increased risk of bleeding, (as defined by the study protocol) that will be included as contraindications in section 4.3 of the SPC (see question 1); close monitoring of reports and serious adverse events during the post-marketing phase as well as data from further studies are needed.

The applicant was asked to provide additional supplementary information for review by the CPMP prior to opinion.

Proposed study protocols to address the following:

a. Any potential drug interaction of co-administered low dose heparin and drotrecogin alfa (activated) in patients with severe sepsis;
b. Further safety and efficacy data should be obtained in sepsis patients at lower risk of death (APACHE II score of 24 or less);
c. Further data on bleeding should be obtained in order to evaluate the incidence of severe/fatal bleeding episodes (intracranial bleedings), incidence of severe/fatal bleeding episodes (overall) and the identification of specific subgroups at higher risk of severe bleeding.

Draft and/or final study protocols of the following post-marketing studies:

- Efficacy and safety of drotrecogin alfa (activated) in approximately 11,350 adult patients with severe sepsis and a lower risk of death (e.g., APACHE II score of 24 or less);
- Efficacy and safety of drotrecogin alfa (activated) in a study of approximately 500 paediatric patients with severe sepsis;
- Effect of low-dose heparin on mortality in a study of approximately 2000 adult patients with severe sepsis who have a high risk of death and are receiving drotrecogin alfa.

In addition, the applicant submitted a cumulative safety review of all serious adverse drug reaction reports which have been reported for drotrecogin alfa (including in open label clinical trials, compassionate use and any post-marketing experience) until 31 January 2002 by 1 March 2002.

Following the review of the submitted documentation, and the final proposed SPC, the CPMP concluded that a marketing authorisation for Xigris will be granted under exceptional circumstances, subject to fulfilling the chemical, pharmaceutical and biological and clinical follow-up measures and clinical specific obligations undertaken by the applicant. Since no other similar product has yet managed to show efficacy compared to placebo in the same indication and only one pivotal study has been performed with Xigris, the CPMP felt that in the present state of scientific knowledge, the applicant cannot be expected to provide comprehensive information on the safety and efficacy of the medicinal product. In order to collect additional long-term data, the applicant has committed to complete a programme of clinical studies post-authorisation within pre-specified time frames, the results of which shall form the basis of an annual re-assessment of the benefit/risk profile.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Xigris in the treatment of adult patients with severe sepsis with multiple organ failure when added to best standard care, was favourable and therefore recommended the granting of the marketing authorisation.