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Briefing Document for
XIGRIS™
for the Treatment of Severe Sepsis

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Executive Summary

Eli Lilly and Company is seeking approval of drotrecogin alfa (activated) for the treatment of adult and pediatric patients with severe sepsis. Information in this document provides an overview of data contained in the Biologics License Application submitted to the United States Food and Drug Administration on 25 January 2001. The proposed indication statement for drotrecogin alfa (activated) reads: *"Drotrecogin alfa (activated) is indicated for the treatment of adult and pediatric patients with sepsis associated with acute organ dysfunction (severe sepsis). Treatment with drotrecogin alfa (activated) reduces mortality in patients with severe sepsis."* Drotrecogin alfa (activated) will be recommended for use as adjunctive therapy to best standard of care in the treatment of patients with severe sepsis.

An estimated 750,000 episodes of severe sepsis occur annually in the Unites States (Linde-Zwirble et al. 1999). Severe sepsis is now the thirteenth most common cause of death in the United States and is among the most common causes of death in the non-coronary intensive care unit (Balk 2000). Few clinical syndromes are associated with such a rapidly progressive clinical course as severe sepsis: between 3 and 5 of every 10 patients die within 4 weeks of diagnosis (Rangel-Frausto et al. 1995; Natanson et al. 1998). This extremely high mortality rate persists despite best standard of care including administration of appropriate antibiotic therapy, adequate source control of infection, and support for failing organs.

The development of severe sepsis is associated with a generalized inflammatory and procoagulant response, as manifested by increased levels of pro-inflammatory cytokines and markers of thrombin generation. These inflammatory and procoagulant host responses are tightly and intricately linked. In spite of adequate antimicrobial therapy and removal of the source of infection, an intensive systemic host response can lead to extensive endothelial damage, fibrin deposition in the microvasculature, organ hypoperfusion, parenchymal cell dysfunction, multiple organ dysfunction, and death.

Activated Protein C, an endogenous protein that promotes fibrinolysis and inhibits thrombosis and inflammation, 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 (Esmon 1989). Reduced levels of Protein C are found in the majority of patients with sepsis and are associated with an increased risk of death. In addition to low plasma levels of Protein C, 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.

The above observations lead to the development of recombinant human Activated Protein C [drotrecogin alfa (activated)] as a therapy for patients with severe sepsis. Data supporting the safety and efficacy of drotrecogin alfa (activated) for the treatment of adult patients with severe sepsis was derived from a single, multi-country, placebo-controlled Phase 3 study F1K-MC-EVAD (n=1690 patients) with supporting data from a

single, multi-country, placebo-controlled Phase 2 study (n=131 patients). Analysis of data from the Phase 2 study F1K-MC-EVAA indicated that drotrecogin alfa (activated), administered as a 24 μg/kg/hr constant rate infusion for 96 hours, reduced markers of coagulopathy (D-dimer) and inflammation (IL-6) compared to placebo. Analysis of data from the Phase 3 study F1K-MC-EVAD indicated that drotrecogin alfa (activated), administered as a 24 μg/kg/hr constant rate infusion for 96 hours, significantly reduced 28-day all cause mortality in patients with severe sepsis compared to placebo (relative risk = 0.8057; p=0.0054). At Study Day 28, the observed mortality rates were 24.71% for the drotrecogin alfa (activated) group and 30.83% for the placebo group. These data represent an unprecedented breakthrough: in the pivotal Phase 3 study F1K-MC-EVAD, an additional 6 lives were saved for every 100 patients treated with drotrecogin alfa (activated). Consistent with its antithrombotic and profibrinolytic effects, the administration of drotrecogin alfa (activated) was associated with an increase in the percent of patients experiencing a bleeding event reported as a serious adverse event (3.5% vs. 2.0%; p=0.06). Serious bleeding events frequently resulted from injury to a blood vessel (traumatic or iatrogenic) or following instrumentation of a highly vascular organ, such as the kidney or lung. There were no other safety concerns associated with the administration of drotrecogin alfa (activated) to patients with severe sepsis.

Data supporting the use of drotrecogin alfa (activated) in the pediatric population (newborn to 17 years) was derived from a single, open-label Phase 1B study which provided pharmacokinetic, pharmacodynamic and safety data for drotrecogin alfa (activated) in pediatric patients with severe sepsis (N=83 patients). Although limited by patient number, analysis of data from the Phase 1B study indicated that the pharmacokinetics, pharmacodynamics (as assessed by serial measures of D-dimer), and safety profile of drotrecogin alfa (activated) were similar between pediatric and adult patients with severe sepsis. As the Phase 1B study in pediatric patients with severe sepsis was an open-label study, the efficacy of drotrecogin alfa (activated) for the treatment of severe sepsis in pediatric patients must be extrapolated from the well-controlled Phase 3 study conducted in adults.

Additional safety and effectiveness data on the use of drotrecogin alfa (activated) for the treatment of severe sepsis in pediatric and adult patients are currently being obtained in a global, open-label study (estimated enrollment at completion of the study = 2,500 patients). The ongoing open-label study employs similar inclusion criteria, exclusion criteria, and dosing regimen as were used in the pivotal Phase 3 study.

The data contained within the BLA demonstrate that drotrecogin alfa (activated) has a favorable benefit risk profile. In the population studied, one additional life was saved for every 16 patients treated with drotrecogin alfa (activated).

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Abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
APACHE II	Acute Physiology and Chronic Health Evaluation II
APC	Activated Protein C
APTT	activated partial thromboplastin time
$\Delta APTT_{max}$	maximum change from baseline in APTT response
AST	aspartate aminotransferase
BLA	Biologics License Application
BT	bleeding time
CI	confidence interval
Cl_p	plasma clearance
C_{ss}	plasma concentration at steady-state
CVVHD	continuous venovenous hemodialysis
DIC	disseminated intravascular coagulation
DSMB	Data Safety Monitoring Board
EPCR	endothelial Protein C receptor
FDA	Food and Drug Administration
F1.2	prothrombin fragment F1.2
HSA	human serum albumin
ICH	International Conference on Harmonisation
ICU	intensive care unit
IL-6	interleukin-6
LLN	lower limit of normal
LOCF	last observation carried forward
M	males
max	maximum value measured
min	minimum value measured
MODS	multiple organ dysfunction syndrome
PAI-1	plasminogen activator inhibitor-1
PC	Protein C
PT	prothrombin time
ΔPT_{max}	maximum change from baseline in PT response
SD	standard deviation
SIRS	systemic inflammatory response syndrome
SOFA	Sequential Organ Failure Assessment
$t_{½}$	half-life in plasma
$t_{½\alpha}$	half-life in plasma during the initial, rapid distribution phase
$t_{½\beta}$	half-life in plasma during the second, slower elimination phase
t_{last}	sampling time of the last quantifiable plasma concentration
TAFI	thrombin activatable fibrinolysis inhibitor

TATc	thrombin-antithrombin complex
ULN	upper limit of normal
V_{ss}	volume of distribution at steady-state

1. Introduction

1.1. Epidemiology of Severe Sepsis

Approximately 750,000 cases of sepsis associated with acute organ dysfunction (severe sepsis) occur annually in the United States (Angus et al. 2001). The mortality rates associated with severe sepsis in the United States range from 28% to 50% and have remained essentially unchanged for several decades (Natanson et al. 1998). Each year, 215,000 deaths are associated with severe sepsis; deaths after acute myocardial infarction occur at approximately an equal rate (Natanson et al. 1998; Angus et al. 2001; Murphy 2000).

1.2. Definition of Severe Sepsis

In 1992 a Consensus Panel of the American College of Chest Physicians and the Society of Critical Care Medicine met to address several issues related to the concepts and terminology associated with the host response to infection (Bone et al. 1992; Wenzel et al. 1996). The central hypothesis for the proposals developed by this Consensus Panel was that the clinical manifestations associated with serious infections were primarily related to the host response to the infection rather than the underlying infectious agent. Furthermore, similar clinical manifestations could also be induced by non-infectious processes that also led to excessive activation of the inflammatory response pathways. The term systemic inflammatory response syndrome (SIRS) was introduced to describe the constellation of clinical manifestations of the systemic inflammatory response to injury. SIRS is defined as the presence of two or more objective signs of systemic inflammation in the absence of evidence of an infectious disease. SIRS may result from a variety of pathologic insults, such as pancreatitis, burns, and trauma.

Objective signs of systemic inflammation include fever, hypothermia, tachycardia, tachypnea, and neutrophilia or neutropenia. These manifestations result from the release of inflammatory mediators (cytokines, eicosanoids, proteases, kinins, etc.) in response to pathologic insults. Excessive release of these inflammatory mediators may result in the development of diffuse capillary injury, parenchymal cell dysfunction, intravascular coagulation with microvascular thrombosis, and multiple organ dysfunction. The term multiple organ dysfunction syndrome (MODS) is used to describe the development of organ dysfunction in patients with SIRS.

When two or more objective signs of systemic inflammation occur in the presence of a known or suspected infection, the term **sepsis** is used rather than SIRS. Sepsis is usually the result of a serious bacterial infection, but may occur in response to other pathogens such as fungi, viruses, and parasites. When an excessive inflammatory response leads to organ dysfunction in patients with sepsis, the term **severe sepsis** is used.

The inflammatory response is normally regulated by a network of endogenous anti-inflammatory mediators, coagulation inhibitors, and fibrinolytic components. These

regulatory systems maintain a state of homeostasis in blood flow, endothelial cell function, and organ function. The progression from sepsis to severe sepsis is associated with loss of this homeostasis. Of note, progression to severe sepsis is associated with marked activation of the coagulation system with depletion of endogenous regulatory components. In addition, after initial activation, the fibrinolytic system becomes relatively inhibited due to an increase in plasminogen activator inhibitor-1 (PAI-1). These changes lead to an unbalanced coagulation system that favors thrombin generation and fibrin clot formation.

1.3. Historical Approach to Therapy of Severe Sepsis

The historical approach to the treatment of sepsis has included antimicrobial therapy and supportive therapies such as vasopressors and ventilatory management. However, even with optimal antimicrobial and supportive therapy, the mortality rate among patients with severe sepsis remained high at 28% to 50%. The failure of conventional therapy to further improve the clinical outcome for these patients led to the evaluation of therapies directed at restoration of homeostasis in the host response to the infection. Following discoveries on the biochemical mechanisms of the inflammatory response in the early 1980s, therapies designed to inhibit the inflammatory response were evaluated in patients with sepsis. Targets of these therapies included pro-inflammatory cytokines, endotoxin, complement, adhesion molecules, and pro-inflammatory metabolic pathways. These clinical trials demonstrated little, if any, positive effect on clinical outcome measured as all-cause mortality, usually at 28 days following start of therapy. The failure of these measures to improve survival has led to exploration of alternative mechanisms to modulate other pathways involved in the pathophysiology of severe sepsis.

1.4. Pathophysiology of Severe Sepsis

The development of severe sepsis is associated with a generalized inflammatory and procoagulant response, as manifested by increased levels of pro-inflammatory cytokines and markers of thrombin generation. These inflammatory and procoagulant host responses are tightly and intricately linked. In spite of adequate antimicrobial therapy and removal of the source of infection, an intensive systemic host response can lead to extensive endothelial damage, fibrin deposition in the microvasculature, organ hypoperfusion, parenchymal cell dysfunction, multiple organ dysfunction, and death.

The association between activation of coagulation and the development of septic shock was delineated more than 30 years ago (Corrigan et al. 1968). Of note, activation of coagulation was found to be independent of the type of infectious microorganism; gram-positive bacteria, gram-negative bacteria, and parasites were all shown to be capable of triggering this response. Subsequently, abnormalities in fibrinolysis, in addition to coagulation, were frequently documented in patients with sepsis (McGilvary and Rotstein 1998; Vervloet et al. 1998; Levi and ten Cate 1999; van Gorp et al. 1999). Our evolving understanding of the host response at the molecular level in the last three decades has

continued to shed new light on the links between infection, inflammation, and hemostasis.

The link between sepsis and activation of coagulation is perhaps most overt in the subset of patients presenting with bruising and skin lesions referred to as purpura fulminans. Skin biopsies from these patients reveal extensive microvascular thrombosis. Of note, the histopathology of these lesions is very similar to the findings in patients with neonatal purpura fulminans, a disorder associated with a congenital absence of Protein C or Protein S, two proteins that are necessary for the regulation of thrombin formation.

Host monocytes and macrophages, in response to infectious pathogens/products, generate and release inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Parrillo 1993). Although these early response cytokines play a critical role in host defense by attracting activated neutrophils to the site of infection, the entry of these cytokines and pathogen products into the systemic circulation can bring about widespread activation of coagulation and suppression of fibrinolysis (van der Poll et al. 1990; van Deventer et al. 1990; Carvalho and Freeman 1994; Hinshaw 1996). The infectious agents and inflammatory cytokines activate coagulation by stimulating the surface expression of tissue factor on monocytes and the endothelium. The exposure of tissue factor to circulating blood initiates coagulation activation that leads to the generation of thrombin and fibrin deposition (clot). For example, in an animal endotoxemia model, it was demonstrated that microthrombi developed in the hepatic microcirculation within 5 minutes of endotoxin challenge (Asaka et al. 1996). If endotoxin exposure continued, multiple fibrin clots developed and resulted in focal areas of hypoperfusion, tissue necrosis, and development of multiple organ dysfunction.

Thrombin is a potent serine protease that has a number of functions, including multiple pro-inflammatory properties (Esmon 2000a). The increased thrombin generation resulting from coagulation activation induced by infectious agents and inflammatory cytokines initiates a vicious cycle by further intensifying the host inflammatory and coagulation response.

As part of the host's attempt to interrupt this vicious cycle and re-establish homeostasis, anti-inflammatory cytokines are released (Bone et al. 1997; Dinarello 1997; Antonelli 1999; van der Poll and van Deventer 1999; Calandra and Heumann 2000; Cavaillon and Adib-Conquy 2000). On the side of hemostasis, the host's endogenous fibrinolytic system and anticoagulant systems are brought into action to try to counter the excessive coagulation activation. Key components of the fibrinolytic system include: tissue plasminogen activator (tPA), which initiates the generation of plasmin; plasminogen, which, when converted to the active enzyme plasmin, is responsible for lysis of fibrin clots; PAI-1, a potent inhibitor of tPA; and thrombin activatable fibrinolysis inhibitor (TAFI) which, when activated by thrombin, suppresses the activity of plasmin (Bazjar 2000). The host's fibrinolytic system is impaired during sepsis by the inflammatory mediators that stimulate the release of PAI-1 from platelets and the endothelium.

Consequently, PAI-1 levels are elevated and tPA activity is suppressed in patients with sepsis (Suffredini et al. 1989; Vervloet et al. 1998).

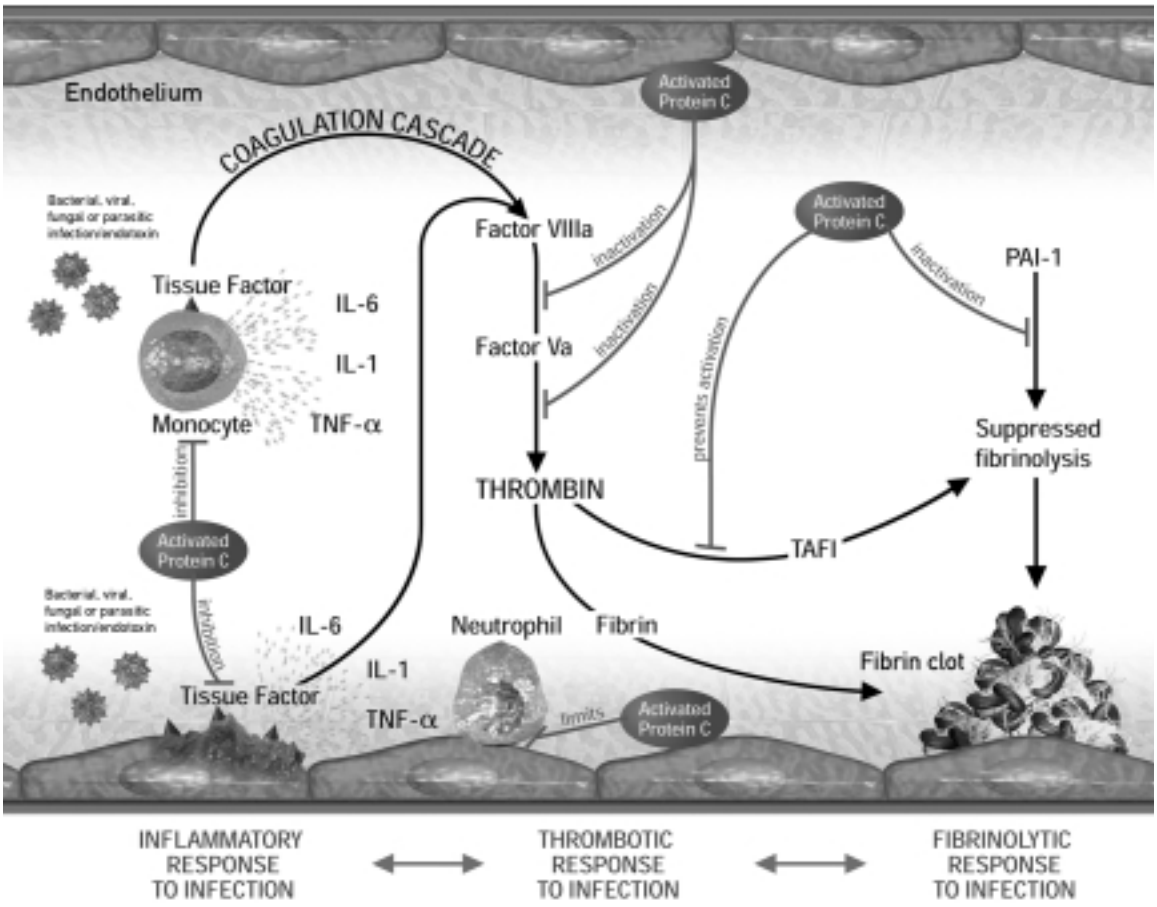


Figure 1.1. **Proposed Actions of Activated Protein C on Inflammation and Coagulation (Bernard et al. 2001; Appendix 5).**

1.5. Mechanisms of Action of Activated Protein C

1.5.1. Antithrombotic Activity

The Protein C pathway is one of three major anticoagulant systems involved in the regulation of thrombin formation (Figure 1.1). Central to the Protein C pathway is the vitamin K-dependent factor Protein C. Protein C is the inactive precursor (zymogen) of the serine protease Activated Protein C. Protein C is converted to Activated Protein C by thrombin in complex with an endothelial surface receptor called thrombomodulin (Esmon 1989). The activation of Protein C is further augmented by another endothelial surface protein, endothelial Protein C receptor (EPCR) (Esmon et al. 1999). Activated Protein C inactivates Factors Va and VIIIa, two key factors in the formation of thrombin. Factor Va accelerates the activation of thrombin by Factor Xa, whereas Factor VIIIa accelerates the activation of Factor X by Factor IXa. Inactivation of Factors Va and VIIIa by

Activated Protein C thus limits the generation of thrombin and is a potent antithrombotic mechanism.

Many studies have shown that Protein C is depleted in both adult and pediatric patients with sepsis and that there is an inverse correlation between the level of Protein C and mortality and morbidity outcomes in these patients (Fourrier et al. 1992; Lorente at al. 1993; Powars et al. 1993; Boldt et al. 2000; Fisher and Yan 2000). From the study reported by Mesters and coworkers, the decrease in plasma Protein C levels preceded the onset of the clinical symptoms of severe sepsis and septic shock by a median of 12 hours, indicating that depletion of Protein C occurs early in the disease course (Mesters et al. 2000). This early decrease in Protein C levels in the pathogenesis of sepsis is further supported by the prevalence (>90%) of acquired Protein C deficiency in patients with severe sepsis (Hartman et al. 1998; Yan et al. 2001). The depletion of Protein C in patients is most probably due to a combination of several mechanisms. Protein C is susceptible to degradation by neutrophil elastase, which is released during sepsis (Philapitsch and Schwartz 1993). The continuous and rapid conversion of Protein C to Activated Protein C in sepsis can lead to depletion of the plasma pool of Protein C. The biosynthesis of Protein C to replenish the circulating pool, a process that is dependent on the liver, may be inadequate due to hepatic dysfunction or acquired vitamin K deficiency.

In addition to the decreased plasma concentration of Protein C, the conversion of Protein C to Activated Protein C may be impaired in patients with severe sepsis. In vitro studies have shown that endotoxin and inflammatory cytokines, such as TNF-α, can down-regulate the endothelial surface thrombomodulin either by decreasing synthesis or by increasing degradation of thrombomodulin (Moore et al. 1987; Moore et el. 1989; Lentz et al. 1991). Endothelial surface thrombomodulin is also cleaved by neutrophil elastase and released into the circulation as soluble thrombomodulin (MacGregor et al. 1997). An elevation of circulating soluble EPCR has been demonstrated in both an experimental mouse sepsis model (Gu et al. 2000) and in patients with sepsis (Kurosawa et al. 1998). Most recently, endothelial surface thrombomodulin and EPCR were shown to be reduced in skin biopsy samples from 21/21 patients and 17/21 patients, respectively, with meningococcal septicemia (Faust et al. 2000). Thus in patients with severe sepsis, even though there is an elevation of thrombin generation, the conversion of Protein C to Activated Protein C may be limited by the combination of decreased plasma concentrations of Protein C and a decrease in the concentration of endothelial surface thrombomodulin and EPCR. This conclusion is supported by data on the levels of Activated Protein C in an animal model of bacteremia (Taylor et al. 2000b) and data from patients with severe sepsis (Mesters et al. 2000), suggesting that the rise in Activated Protein C in sepsis is transient and does not parallel with the continuous rise in thrombin levels.

In addition to impairment of the Protein C system, the plasma concentration of antithrombin is reduced in patients with severe sepsis. The decrease in antithrombin also appears to occur early in the disease process. Overall, the data suggest that hemostasis is

unbalanced in patients with severe sepsis, with an increase in coagulation activation and thrombin generation and impairment of the hemostatic regulatory systems. This imbalance contributes to enhancement of the inflammatory response, microvascular hypoperfusion, organ dysfunction, and the high mortality in patients with severe sepsis.

1.5.2. Profibrinolytic Activity

Activated Protein C has been shown to have profibrinolytic activity in an animal model (Jackson et al. 1998). The profibrinolytic activity of Activated Protein C is an indirect effect mediated by three possible molecular mechanisms. First, Activated Protein C inhibits PAI-1. This inhibition involves the formation of a stable complex between PAI-1 and Activated Protein C. PAI-1 trapped in this complex is not capable of inhibiting tPA. Consequently, high levels of Activated Protein C may result in less inhibition of tPA because of the competition for PAI-1. Second, in vitro data suggest that Activated Protein C may inhibit the release of PAI-1 from endothelial cells, again resulting in less inhibition of tPA. Third, activation of TAFI is dependent on high concentrations of thrombin. Inhibition of thrombin generation by Activated Protein C thus limits the activation of TAFI (Bazjar et al. 1996).

1.5.3. Anti-Inflammatory Activity

The proposed anti-inflammatory activities of Activated Protein C, a more recent discovery, are based mostly on in vitro data (Grinnell and Yan 1998). Since thrombin has been shown to have multiple pro-inflammatory activities, limiting thrombin generation by Activated Protein C would have indirect anti-inflammatory effects (Esmon 2001). In addition, Activated Protein C has been show to directly interact with endothelial cells and leukocyte membranes in combination with EPCR (Esmon 2000b). This Activated Protein C:EPCR membrane complex has been shown to alter cell responses. Activated Protein C reduces NFκB nuclear translocation resulting in reduction of cytokine synthetic rates (Esmon 2000c). Activated Protein C decreases the expression of leukocyte adhesion molecules on the surface of endothelial cells, resulting in decreased interaction between these cells, a key step in the movement of leukocytes out of the vessels and into the tissues. Anti-apoptotic pathways are activated following exposure of cells to Activated Protein C; activation of these pathways is associated with decreased apoptosis in response to the apoptotic agent staurosporin (Joyce et al. 2001). The molecular mechanism for these effects is still uncertain, but may involve translocation of Activated Protein C to the nucleus of inflammatory cells, affecting the transcriptional activity of acute phase response genes (White et al. 2000).

1.6. Rationale of Testing Activated Human Protein C as Therapeutic Agent in Severe Sepsis

In view of the role of unbalanced activation of coagulation in the pathophysiology of severe sepsis, a molecule such as Activated Protein C, with its multiple proposed

mechanisms of action, is an attractive candidate for clinical evaluation. Activated Protein C may help break the vicious cycle of the host response, leading to improvement in organ function and survival.

Experiments with the baboon model of bacteremia provide additional support for the potential role of administration of Activated Protein C to patients with severe sepsis (Taylor et al. 1987; Taylor et al. 2000a). In one series of experiments, all control animals receiving a lethal dose of bacteria died of sepsis-related complications, whereas all of the animals that received the same dose of bacteria in conjunction with administration of Activated Protein C survived. Administration of Activated Protein C was also associated with decreased thrombin generation and amelioration of the coagulopathy in the septic animals, as monitored by a variety of biomarkers.

Subsequent experiments explored the role of EPCR in the host response to sepsis. Animals were given either a control antibody or an antibody that blocked the interaction between EPCR and Protein C, followed by a sublethal dose of bacteria. All animals receiving the control (non-inhibitory) antibody survived, whereas all animals that received the inhibitory antibody died of sepsis-related complications. The fatal outcome in the animals receiving the inhibitory antibody was presumed to be due to decreased activation of Protein C. In another series of experiments, baboons were treated with the control antibody or the inhibitory antibody and then infused with thrombin to stimulate activation of Protein C. Administration of the blocking antibody to EPCR inhibited Protein C activation by 88% (Taylor et al. 2001). Thus, inhibition of EPCR function leads to decreased activation of Protein C and poorer outcomes in this model of sepsis.

Taken together, these animal model data provide additional evidence supporting the hypothesis that Activated Protein C may be effective at reducing mortality among patients with severe sepsis. In addition, these data demonstrate the importance of an intact endothelial system for generation of Activated Protein C in vivo. The observation that endothelial expression of both EPCR and thrombomodulin are suppressed in patients with severe sepsis suggests that the endogenous system for generating Activated Protein C is diminished. Administration of the activated form of Protein C bypasses this defect associated with sepsis because the active enzyme does not rely on the host's endothelial thrombomodulin or EPCR for conversion. For this reason, the activated form of Protein C is preferred over the precursor (zymogen) form for the treatment of patients with severe sepsis.

1.7. Early Development of Activated Protein C as a Potential Therapeutic Agent for Patients with Severe Sepsis

1.7.1. Drotrecogin Alfa (Activated) (Recombinant human Activated Protein C)

The plasma concentration of Activated Protein C (1 to 2 ng/mL) and zymogen Protein C (approximately 4000 ng/mL) in healthy humans is too low to support large scale production of Activated Protein C from the human blood supply. Consequently, recombinant DNA technology is used by the sponsor to produce human Activated Protein C. The proposed generic name for the recombinant human Activated Protein C produced by the sponsor's process is drotrecogin alfa (activated).

Human Protein C was cloned and a suitable expression system developed (Beckmann et al. 1985; Grinnell et al. 1987; Yan et al. 1990). Because of the very complex structure of human Protein C requiring four different types of post-translational modifications for full biological activity, recombinant human Activated Protein C has to be produced using a mammalian cell line (Grinnell et al. 1987; Yan et al. 1990). The recombinant molecule is identical to the plasma-derived human Activated Protein C in its protein sequence. It is distinguished only by differences in the carbohydrate portion of the molecule (Yan et al. 1993).

1.7.2. Preclinical Toxicology

The results of the baboon studies indicated a potential mortality benefit of Activated Protein C for sepsis (Section 1.6). In support of the clinical program, an extensive series of pharmacodynamic, pharmacokinetic, and toxicology studies were conducted with drotrecogin alfa (activated). The only adverse event reported in these studies was bleeding. These bleeding events were generally categorized as nonserious and involved bleeding or oozing at venipuncture or surgical sites. Spontaneous, nontraumatic hemorrhage was uncommon. The preclinical toxicology studies of drotrecogin alfa (activated) provided supporting data for the initial clinical development program of drotrecogin alfa (activated) to a maximum infusion rate of 50 μg/kg/hr.

1.8. Summary

The mortality of severe sepsis remains at 30% to 50% in spite of advances in antimicrobial therapies and life support modalities. Severe sepsis is manifested with a systemic host response of inflammation and coagulopathy that leads to endothelial injury, fibrin deposition, hypoperfusion, multi-organ dysfunction, and death. The multiple mechanisms of action of Activated Protein C (antithrombotic, profibrinolytic, and anti-inflammatory) and the safety profile of drotrecogin alfa (activated) from preclinical toxicology studies support the evaluation of this compound as a treatment of severe sepsis in improving survival. The learning and experience gained from past clinical trials

in sepsis in the last two decades have been invaluable in shaping the design of the clinical trial protocols for this program.

2. Overview of Clinical Studies

Drotrecogin alfa (activated) has been studied in a variety of patient populations, including healthy subjects and patients with end-stage renal disease (Phase 1), as well as adult and pediatric patients with severe sepsis (Phase 1B and Phase 2/3). The information presented in this section contains an overview of each of these studies including study design, objectives, patient population, dose duration, and results.

Completed Phase 1/1B Studies

Eight Phase 1 studies were completed and included in the BLA (Table 2.1). The primary objective of the initial seven studies of the Phase 1 program was to evaluate the safety of drotrecogin alfa (activated) in healthy male and female subjects who received doses up to 48 µg/kg/hr for durations up to 24 hours. An additional study evaluated safety in male and female subjects with end-stage renal disease receiving either hemodialysis or peritoneal dialysis.

Table 2.1. Phase 1 Completed Clinical Studies

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-GUAA One	Open-label	Healthy male adult subjects	To evaluate safety as assessed by APTT, PT, CBC, platelet count, occult fecal blood, serum chemistry, urinalysis, and anti-APC antibody testing, plasma APC levels	4 subjects 4 male 26 to 49 years	Drotrecogin alfa (activated): 0.49 to 25.7 µg/kg/hr 3-hr infusion	Drotrecogin alfa (activated) was well-tolerated at administered dose/duration. Cp_{ave} and $AUC_{0-\infty}$ increased proportionally with infusion rate. $t_{½}$ ranged from 0.124 to 0.930 hrs.
F1K-LC-GUAB One	Open-label	Healthy adult subjects	Evaluation of APTT, PT, BT, F1.2, FPA, Protein C antigen and activity, anti-APC antibody assay, plasma APC levels, platelet aggregation, D-dimer	4 subjects 4 male 29 to 48 years	Drotrecogin alfa (activated): 6.04 to 49.1 µg/kg/hr 3-hr infusion	Drotrecogin alfa (activated) was well-tolerated at administered dose/duration. Cp_{ave} and $AUC_{0-\infty}$ increased proportionally with infusion rate. $t_{½}$ ranged from 0.487 to 1.97 hrs. Plasma drotrecogin alfa (activated) concentration correlated with % change in baseline APTT values; Platelet inhibition was not observed.

(continued)

Table 2.1. Phase 1 Completed Clinical Studies (continued)

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-GUAC One	Open-label	Healthy adult subjects	Evaluate APTT, PT, BT, F1.2, FPA, Factor V, Factor VIII, Protein C antigen and activity, anti-APC antibody assay, plasma APC levels, platelet aggregation, D-dimer	32 subjects 11 male 21 female 43 to 76 years	Drotrecogin alfa (activated): 6.59 to 24.2 μg/kg/hr 6- and 24-hr infusion	Drotrecogin alfa (activated) was well-tolerated at administered dose/duration; Cp_{ave} and $AUC_{0-\infty}$ increased proportionally to infusion rate. Cl_p was independent of gender and estrogen status. Individual $t_{½}$ ranged from 0.0584 to 1.88 hrs. PT and bedside APTT values correlated highly with serum drotrecogin alfa (activated) concentrations; platelet function not inhibited; no anti-drotrecogin alfa (activated) antibody formation.

Table 2.1. Phase 1 Completed Clinical Studies (continued)

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-GUAD One	Open-label	Healthy adult subjects, including postmenopausal females with and without supplemental estrogen use	Evaluate APTT, PT, BT, anti-APC antibody assay, plasma APC levels, platelet aggregation, Factor V, Factor VIII, and APC inhibitors	51 subjects 18 male 33 female 40 to 78 years	Drotrecogin alfa (activated): 12.8 to 49.9 µg/kg/hr 6- and 24-hr infusion	Drotrecogin alfa (activated) was well tolerated. C_{ss} was proportional to infusion rate but independent of infusion duration. Cl_p was comparable in women with estrogen and in men, but was lower in women without estrogen than in men. Harmonic mean $t_{1/2}$ ranged from 0.4 to 1.9 hours at infusion rates of 12 to 48 µg/kg/hr for 24 hrs. Drotrecogin alfa (activated) produced a dose-proportional increase in bedside $\Delta APTT_{max}$ and, to a much lesser extent, in $\Delta PT_{max.}$

Table 2.1. Phase 1 Completed Clinical Studies (continued)

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-GUAE One	Open-label	Subjects with end-stage renal disease; (hemodialysis and peritoneal dialysis)	Evaluate APTT, PT, BT, platelet aggregation, plasma APC levels, fibrinopeptide A, F1.2, D-dimer, and APC inhibitors	13 subjects 7 male 6 female 21 to 68 years	Drotrecogin alfa (activated): 26.3 μg/kg/hr 6-hr infusion	No significant changes in vital signs, safety laboratory tests, or BT. Mean Cl_p was 23.1 and 29.8 L/hr for peritoneal dialysis patients and hemodialysis patients, respectively.
F1K-LC-GUAF One	Part A: Aspirin alone; Part B: a crossover design, single-blind study comparing drotrecogin alfa (activated) in the presence of aspirin or placebo	Healthy adult subjects	Evaluate APTT, PT, BT, platelet aggregation, plasma APC levels, and anti-APC antibody assay	Part A: 15 subjects 9 male 6 female 41 to 61 years Part B: 27 subjects 11 male 16 female 40 to 75 years	Part A: Enteric-coated aspirin (500 mg) orally Part B: (1) Enteric-coated aspirin (500 mg) orally followed by drotrecogin alfa (activated) 25.1 μg/kg/hr 6-hr infusion or (2) Placebo (orally) followed by drotrecogin alfa (activated) 25.1 μg/kg/hr (1 mg/m^2/hr) 6-hr infusion	Drotrecogin alfa (activated) was well tolerated. Aspirin pretreatment had no effect on drotrecogin alfa (activated) pharmacokinetics and PT or bedside APTT.

Table 2.1. Phase 1 Completed Clinical Studies (continued)

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-EVAK One	Open-label	Healthy adult subjects	Evaluate APTT, PT, BT, plasma APC levels following bolus (Part A) and loading doses (Part B)	Part A: 6 subjects 6 male 35 to 48 years Part B: 6 subjects 6 male 40 to 61 years	Drotrecogin alfa (activated): Part A Subjects received the following: 1) Up to 10 µg/kg over 1 min, or 2) 12 µg/kg/hr for 6 hr, or 3) Combined 1 and 2 Part B 1) 12.3 µg/kg/hr (average infusion rate over 2 infusions) or 2) 12.8 µg/kg/hr (average infusion rate over 3 infusions)	Drotrecogin alfa (activated) was well tolerated at administered dose/duration. Bedside APTT did not exceed 2 times baseline values and correlated with plasma drotrecogin alfa (activated) concentration. The two- and three-step infusions produced quicker attainment of C_{ss} than did the single-rate infusion.

Table 2.1. Phase 1 Completed Clinical Studies (concluded)

Study/ Number of Centers	Design	Study Population	Objective	Subjects: Number Entered/ Gender/ Age Range	Dose Duration	Key Results
F1K-LC-EVAM One	Open-label	Healthy adult subjects including post-menopausal females	Evaluate APTT, PT, BT, anti-APC antibodies, and plasma APC levels following a loading dose	14 subjects 8 male 6 female 29 to 67 years	Drotrecogin alfa (activated): 1) 12.5 µg/kg/hr (average infusion rate over 2 infusions), or 2) 24.7 µg/kg/hr (average infusion rate over 2 infusions), or 3) 49.8 µg/kg/hr (average infusion rate over 2 infusions)	Drotrecogin alfa (activated) was well tolerated. No clinically significant alterations in laboratory values. Bedside APTT during the initial 30-min loading infusion did not exceed 3 times baseline value. Cl_p and C_{ss} were consistent with those measured during constant rate infusions.

Abbreviations: APC = Activated Protein C; APTT = bedside whole blood activated partial thromboplastin time; $\Delta APTT_{max}$ = change from baseline in maximum APTT response; $AUC_{0-\infty}$ = area under the concentration-time curve through infinity; BT = template bleeding time; CBC = complete blood count; Cl_p = plasma clearance; Cp_{ave} = average plateau plasma clearance; C_{ss} = constant steady-state concentration; F1.2 = prothrombin fragment F1.2; FPA = fibrinopeptide A; PT = bedside whole blood prothrombin time; ΔPT_{max} = change from baseline in maximum PT response; $t_{½}$ = half-life.

Source: Completed Study Reports for Studies F1K-LC-GUAA, F1K-LC-GUAB, F1K-LC-GUAC, F1K-LC-GUAD, F1K-LC-GUAE, F1K-LC-GUAF, F1K-LC-EVAK, and F1K-LC-EVAM.

Ongoing Phase 1B Study

Study F1K-MC-EVAO is an open-label, dose-escalation study that is currently ongoing at 12 sites in the United States and the United Kingdom. This study is designed to investigate the pharmacokinetics and safety of drotrecogin alfa (activated) administered to pediatric patients with severe sepsis. Additionally, the pharmacodynamics of drotrecogin alfa (activated), as assessed by changes in plasma coagulation parameters (D-dimer concentration, Protein C activity level, and antithrombin activity level), will be investigated. In Part 1, infusions of drotrecogin alfa (activated) administered at 6, 12, 24, and 36 μg/kg/hr for 6 hours once daily over a 4-day treatment period in three age groups (newborn to <1 year, ≥1 year to <8 years, and ≥8 years to <18 years) were evaluated for an appropriate dose for Part 2. In Part 2, patients received a 96-hour infusion at 24 μg/kg/hr (based on Part 1 results) (Table 2.2).

Table 2.2. Phase 1B Ongoing Clinical Studies

Study Number of Centers	Design	Study Population	Objective	Patients Gender	Dose Duration
F1K-MC-EVAO 12	Open-label	Pediatric patients with severe sepsis	Investigate the pharmacokinetics and safety of drotrecogin alfa (activated)	Part 1: 21 patients 10 males 11 females Part 2: 62 patients 32 males 30 females	Part 1: infusion of drotrecogin alfa (activated) administered at 6, 12, 24, and 36 μg/kg/hr for 6 hr once daily over 4 days Part 2: 96-hr infusion at 24 μg/kg/hr (based on Part 1 results)

Source: Protocol for Study F1K-MC-EVAO and Data Capture up to 31 May 2001.

Phase 2/3 Studies

Drotrecogin alfa (activated) has been evaluated in the treatment of severe sepsis in two randomized, double-blind, placebo-controlled, multicenter clinical studies (Table 2.3).

Study F1K-MC-EVAA was a Phase 2 dose-ranging study conducted at 40 investigative sites in the United States and Canada. The primary objectives of this study were to evaluate the safety of administration of drotrecogin alfa (activated) as a function of infusion rate and infusion duration and the degree to which the coagulation abnormalities of severe sepsis were affected by the administration of drotrecogin alfa (activated) as a function of infusion rate and infusion duration; and to determine an effective infusion rate and infusion duration of drotrecogin alfa (activated) administration based on its ability to alter the coagulation abnormalities of severe sepsis. The study was divided into two stages. During Stage 1, patients were randomly assigned to receive placebo or drotrecogin alfa (activated) at an infusion rate of 12, 18, 24, or 30 μg/kg/hr administered for a 48-hour infusion duration. During Stage 2, patients were randomly assigned to receive placebo or drotrecogin alfa (activated) at an infusion rate of 12, 18, or 24 μg/kg/hr administered for a 96-hour infusion duration. All patients were followed for 28 days or until death. Of the 135 patients with severe sepsis enrolled in the study, 131 received study drug: 4 patients withdrew from the study before receiving any study drug, 90 patients received drotrecogin alfa (activated), and 41 patients received placebo.

Study F1K-MC-EVAD was a Phase 3 pivotal efficacy and safety trial conducted at 164 investigative sites in 11 countries (Australia, Brazil, Belgium, Canada, France, Germany, Netherlands, New Zealand, Spain, South Africa, and the United States) in 1728 randomly assigned patients with severe sepsis. Of these patients, 1690 received study drug (850 [drotrecogin alfa (activated)], 840 placebo). This study compared infusion of drotrecogin alfa (activated) administered at 24 μg/kg/hr with infusion of placebo for 96 hours. The primary objective of the study was to demonstrate a reduction in 28-day all-cause mortality with treatment of drotrecogin alfa (activated) compared with placebo.

Table 2.3. Phase 2/3 Clinical Studies

Study Number of Centers	Design	Study Population	Primary Objective	Number of Patients Gender Age Range	Drotrecogin Alfa (Activated) Dose Duration	Key Results
F1K-MC-EVAA 40	Randomized, double-blind, placebo-controlled, multicenter, dose-ranging	Adult patients with severe sepsis	Safety of drotrecogin alfa (activated) as a function of dose and duration	Enrolled=135 Primary Analysis Population=131 84 male 47 female 19 to 89 years	Dose-ranging 48-hr infusion (12, 18, 24, or 30 μg/kg/hr) or 96-hr infusion (12, 18, or 24 μg/kg/hr)	Improvement of sepsis-induced coagulation abnormalities (D-dimer, and IL-6 levels). Acceptable safety profile. Recommended infusion rate and duration of 24 μg/kg/hr for 96 hr.
F1K-MC-EVAD 164	Randomized, double-blind, placebo-controlled, multicenter	Adult patients with severe sepsis	Evaluate 28-day all-cause mortality	Enrolled=1728 Primary Analysis Population=1690 964 male 726 female 18 to 96 years	24 μg/kg/hr for 96 hr	19.43% decrease in the relative risk of death, a 27.59% decrease in the odds of death, and a 38.1% increase in the odds of survival were observed in drotrecogin alfa (activated) patients compared with placebo patients.

Abbreviations: IL-6 = Interleukin 6.
Source: Completed Study Report for Studies F1K-MC-EVAA and F1K-MC-EVAD.

3. Pharmacokinetics and Safety in Healthy Subjects

Phase 1 Studies. The clinical pharmacology and pharmacokinetics of drotrecogin alfa (activated) were evaluated in eight Phase 1 studies. These studies, comprising 112 unique subjects, are summarized in an integrated fashion.

The primary objective of the Phase 1 program was to evaluate the safety of drotrecogin alfa (activated) over a range of doses up to 48 μg/kg/hr for 24 hours. Phase 1 studies were conducted in healthy males and females and in: (a) healthy females with low estrogen levels, (b) subjects undergoing hemodialysis or peritoneal dialysis as therapy for end-stage renal disease, and (c) healthy males and females pretreated with aspirin. Of the 172 subjects entered in Phase 1 studies, 27 were not exposed to drotrecogin alfa (activated) and 33 participated in two to four study protocols.

The safety profile of drotrecogin alfa (activated) in the Phase 1 studies was evaluated by measuring the effect of drotrecogin alfa (activated) on the following physiological parameters:

- Bedside whole blood activated partial thromboplastin time (APTT)
- Template bleeding time (BT)
- Bedside whole blood prothrombin time (PT)
- Factor V and Factor VIII
- Complete blood count
- Platelet count
- Occult fecal blood and occult urine blood
- Serum chemistries
- Electrolytes
- Urinalysis
- Spontaneous and solicited adverse events
- Anti-APC antibody formation.

Conventional noncompartmental and compartmental methods were used to calculate the following primary pharmacokinetic parameters in Phase 1 studies:

- Plasma concentration at steady-state (C_{ss})
- Plasma clearance (Cl_p)
- Volume of distribution at steady-state (V_{ss})
- Half-life in plasma ($t_{1/2}$)

3.1. Bioanalytical Method

Plasma concentrations of APC were measured using an immunocapture-amidolytic activity assay specific for APC (Gruber and Griffin 1992). As Activated Protein C is inhibited by several plasma protease inhibitors (Heeb et al. 1989; Marlar et al. 1993; Scully et al. 1993), blood samples were collected into citrate tubes containing benzamidine, a reversible inhibitor of Activated Protein C that prevents inhibition by the plasma protease inhibitors. Benzamidine inhibition was reversed after immunocapture of Activated Protein C and removal of the plasma protease inhibitors, thus restoring the amidolytic activity of Activated Protein C. The assay has a validated range of 1 to 10 ng/mL at the low end and 100 to 200 ng/mL at the high end. Samples with values above the upper limit of quantitation were diluted and re-analyzed.

Pharmacokinetic data of drotrecogin alfa (activated) were derived using the immunocapture assay method, and thus reflect the plasma concentration of active enzyme. The assay method does not distinguish between drotrecogin alfa (activated) and endogenous Activated Protein C. However, the concentration of endogenous Activated Protein C is generally below the lower limit of quantitation. The assay method also does not provide data on the plasma clearance of Activated Protein C-protease inhibitor complexes or Activated Protein C/drotrecogin alfa (activated) metabolites.

3.2. Pharmacokinetics of Drotrecogin Alfa (Activated)

Throughout the descriptions of Phase 1 results, the term "normal healthy subjects" identifies a subset of Phase 1 subjects that excludes estrogen-deficient women, subjects requiring hemodialysis, subjects requiring peritoneal dialysis, and bolus-dosed subjects. These subjects were omitted from the "normal healthy" database because estimates of Cl_p in these subjects were statistically significantly different from those in normal healthy subjects in at least one Phase 1 study (estrogen-deficient women and subjects requiring hemodialysis), because the population studied was not healthy (subjects requiring hemodialysis or peritoneal dialysis), or because parameter estimates were either not calculable or unreliable because steady-state was not attained (bolus-dosed subjects).

Estimates of C_{ss} and Cl_p were robust and were not greatly affected by excluding specific subjects. However, excluding estrogen-deficient women, subjects requiring hemodialysis, subjects requiring peritoneal dialysis, and bolus-dosed subjects preserved the integrity of the database attributed to the normal healthy population, and ensured that parameter estimates ascribed to that population were based on data from subjects who truly were "normal" and "healthy."

Half-Life of Drotrecogin Alfa (Activated) in Healthy Subjects. Elimination of drotrecogin alfa (activated) was biphasic and rapid. Plasma $t_{1/2}$ was 0.913 ± 0.679 hr (N=251 doses) based on all Phase 1 subjects and 0.693 ± 0.411 hr (N=78 doses) based on the more homogenous subset of normal healthy subjects who received drotrecogin alfa (activated) at a dose of 25.1 ± 2.0 μg/kg/hr (Table 3.1). Because of assay sensitivity at

lower doses, these half-life estimates were hybrids of $t_{1/2}$ measured during the initial and terminal phases, and depended on infusion rate and duration. However, the estimates in all subjects and in normal healthy subjects are comparable, which indicates that the overall estimate of $t_{1/2}$ obtained from Phase 1 subjects is robust and is not substantially affected by choice of subject subpopulation.

Because $t_{1/2}$ is so short, C_{ss} is reached rapidly after starting a constant rate infusion. Elimination of drotrecogin alfa (activated) is biphasic, with a rapid initial phase ($t_{1/2\alpha}$) of 13 minutes and a slower second phase ($t_{1/2\beta}$) of 1.63 hours. The short $t_{1/2\alpha}$ of 13 minutes accounts for 79% of the area under the plasma concentration curve, and governs the initial rapid accrual of plasma APC concentrations toward steady-state levels. The longer $t_{1/2\beta}$ of 1.6 hours controls the time it takes to get from 90% of steady-state to 100% of steady-state, and governs the time it takes to eliminate the final 21% of drotrecogin alfa (activated) infused during treatment. Approximately 75%, 90%, and 97% of C_{ss} will be reached within 40 minutes, 1.8 hours, and 4.5 hours, respectively, of starting an infusion. Likewise, those are the fractions of drug that will be eliminated within those times after stopping an infusion. Therefore, the short $t_{1/2}$ of drotrecogin alfa (activated) confers rapid attainment to steady-state during infusion and then rapid elimination after infusion.

Steady-State Plasma Concentration of Drotrecogin Alfa (Activated) in Healthy Subjects. Plasma concentrations of endogenous APC in healthy subjects were usually below detection limits and did not significantly influence the pharmacokinetics of drotrecogin alfa (activated). Steady-state plasma concentrations were proportional to infusion rate in all studies in which infusion rate varied (Figure 3.1). Steady-state plasma concentrations did not depend on infusion duration when infusions at one rate were given to the same subjects for 6 hours and 24 hours (Studies F1K-LC-GUAC and F1K-LC-GUAD).

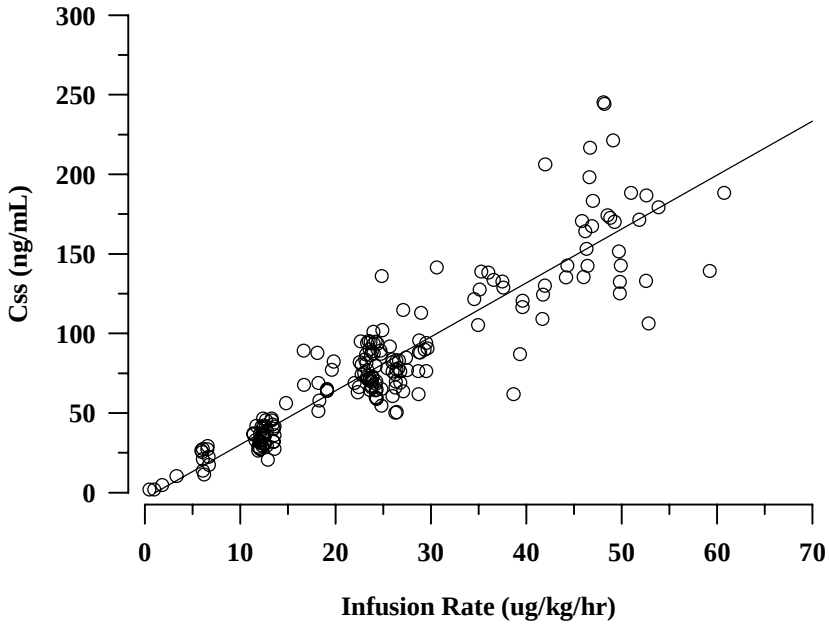


Figure 3.1. **Steady-state plasma APC concentration versus drotrecogin alfa (activated) infusion rate in all drotrecogin alfa (activated) infusions given to normal healthy subjects during Phase 1 studies (N=190 doses).**

Normal healthy subjects exclude estrogen-deficient women, subjects requiring hemodialysis, subjects requiring peritoneal dialysis, and bolus-dosed subjects. Solid line derived by unweighted linear regression. Source: Figure 2 of BLA Section 6.

Based on integrated results from the Phase 1 studies (Table 3.1), C_{ss} at an infusion rate of 24 μg/kg/hr is expected to be 71.2 ng/mL based on all subjects, 68.9 ng/mL based on normal healthy subjects, and 72.4 ng/mL based on normal healthy subjects infused at 25.1 ± 2.0 μg/kg/hr.

Table 3.1. Steady-State Plasma APC Concentrations, Plasma Clearance, Steady-State Volume of Distribution, and Elimination Half-Life During Phase 1 Clinical Trials

Subjects	C_{ss} (ng/mL) Actual	C_{ss} (ng/mL) Normalized to 24 µg/kg/hr[a]	Cl_p (L/hr)	V_{ss} (L)	$t_{1/2}$ (hr)
All subjects (N=251 doses)	NA	71.2 ± 18.6	27.0 ± 8.4	20.3 ± 11.3	0.913 ± 0.679
Normal healthy subjects (N=190 doses)[b]	NA	68.9 ± 15.3	28.1 ± 8.6	19.7 ± 11.5	0.852 ± 0.628
Normal healthy subjects infused at 24 µg/kg/hr (N=78 doses)[b]	79.0 ± 14.9[c]	72.4 ± 15.2	26.0 ± 6.8	17.6 ± 12.8	0.693 ± 0.411

Results are expressed as mean ± SD.

Abbreviations: Cl_p = plasma clearance; C_{ss} = steady-state plasma APC concentration; N = number of doses; NA = not applicable; $t_{1/2}$ = elimination half life; V_{ss} = volume of distribution.

[a] Normalized to 24 µg/kg/hr: Normalized C_{ss} = Actual C_{ss} • $(^{24\ \mu g/kg/hr}/_{\text{Actual infusion rate}})$.

[b] Normal healthy subjects exclude estrogen-deficient women, subjects requiring hemodialysis, subjects requiring peritoneal dialysis, and bolus-dosed subjects for reasons summarized above.

[c] At an actual infusion rate of 25.1 ± 2.0 µg/kg/hr.

Source: Biologic License Application Section 6, Table 3.

Clearance of Drotrecogin Alfa (Activated) in Healthy Subjects. Pharmacokinetic data produced during drotrecogin alfa (activated) development indicate the following:

- Cl_p of drotrecogin alfa (activated) in healthy subjects does not depend on infusion rate or infusion duration.
- Cl_p of drotrecogin alfa (activated) in healthy subjects tends to increase with increasing body weight, thus justifying body weight-normalized dosing in healthy subjects.
- The overall estimate of Cl_p in healthy subjects is robust, in spite of various subject characteristics that influenced estimates of drotrecogin alfa (activated) Cl_p in specific Phase 1 studies.

Based on integrated results from the Phase 1 studies (Table 3.1), estimated Cl_p (mean ± SD) was 27.0 ± 8.4 L/hr (N=251 doses) based on all doses, 28.1 ± 8.6 L/hr (N=190 doses) based on normal healthy subjects, and 26.0 ± 6.8 L/hr (N=78 doses) based on normal healthy subjects infused at 25.1 ± 2.0 µg/kg/hr.

Volume of Distribution of Drotrecogin Alfa (Activated) in Healthy Subjects. The V_{ss} of drotrecogin alfa (activated) is small, which is consistent with the high molecular weight

of drotrecogin alfa (activated) and the presumed effect of that bulk on its ability to penetrate membranes. The V_{ss} of approximately 16 to 20 L in normal healthy subjects is comparable to that of extracellular volume.

3.3. Pharmacokinetic/Pharmacodynamic Relationships

Activated Partial Thromboplastin Time. APTT was the predominant pharmacodynamic parameter assessed during the Phase 1 studies. Drotrecogin alfa (activated) is rapidly neutralized by endogenous plasma protease inhibitors; therefore, the determination of APTT and PT were measured by an assay performed at the patient's bedside using whole blood. The assay was performed within two minutes of the sample being obtained from the subject. Use of whole blood minimized the time necessary to prepare the sample for measurement of APTT and PT. Conduct of the assay at the bedside eliminated the time necessary to transport the sample to the hospital laboratory.

APTT correlated strongly with APC concentration in all individual studies. This relationship is best exemplified by Study F1K-LC-GUAD, in which men and women were infused with drotrecogin alfa (activated) at a mean rate of 12.8, 25.4, 38.0, and 49.9 μg/kg/hr for 6 hours and 24 hours. During these infusions, the time course of the pharmacodynamic response to drotrecogin alfa (activated), expressed as percent change in APTT from baseline (%ΔAPTT), exactly paralleled the time course of drotrecogin alfa (activated) in plasma (Figure 3.2), and showed no evidence of hysteresis. As expected from this relationship, a plot of %ΔAPTT versus plasma APC concentration is linear (Figure 3.3).

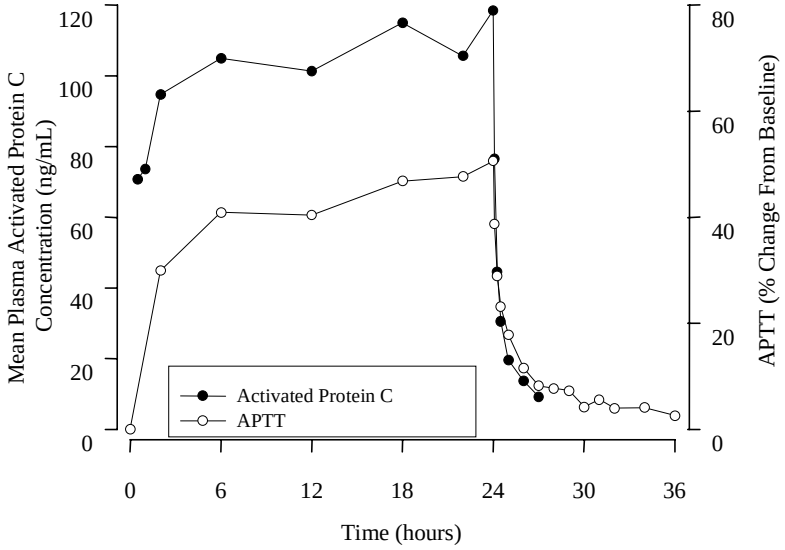


Figure 3.2. **Mean plasma APC concentration and plasma %ΔAPTT versus time during a 24-hour infusion of drotrecogin alfa (activated) at a rate of 25.4 μg/kg/hr during Study F1K-LC-GUAD (N=11 subjects). Source: Figure GUAD.11.3**

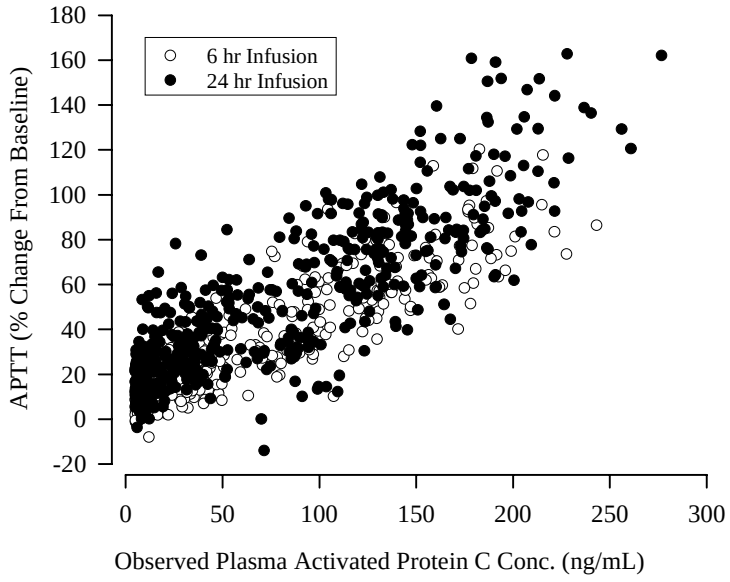


Figure 3.3. **%ΔAPTT versus plasma APC concentration during 6-hour and 24-hour infusions of drotrecogin alfa (activated) at rates of 12.8, 25.4, 38.0, and 49.9 μg/kg/hr during Study F1K-LC-GUAD. Source: Figure GUAD.11.4.**

Consistent with the rapid decline of APC plasma concentrations after the end of infusion, the APTT at the highest drotrecogin alfa (activated) infusion rate and duration of 48 μg/kg/hr for 24 hours returned to within 20% of its predose levels within 5 hours. APTT returned to within 10% of its predose levels within 4 hours after a 24-hour infusion at 24 μg/kg/hr. These times to return to baseline reflect the time it takes for drotrecogin alfa (activated) activity to effectively dissipate.

Prothrombin Time. PT was a secondary pharmacodynamic parameter monitored during Phase 1 studies. PT correlated weakly but statistically significantly with APC concentration in all individual studies. As exemplified by Study F1K-LC-GUAD, the maximal change in PT from baseline (ΔPT_{max}) depended on infusion rate ($p<0.001$) and, at the higher doses, also depended on infusion duration ($p=0.002$). Although the observed changes in PT were statistically significant, the difference was less than 3 seconds. In contrast to the proportional response of APTT to C_{ss}, PT changed little over a 4-fold range of drotrecogin alfa (activated) infusion rates.

Bleeding Time. In Study F1K-LC-GUAD, there was an effect of drotrecogin alfa (activated) on bleeding time that was dependent on estrogen status ($p=0.028$). The absolute difference was only 1 to 1.5 minutes over the entire dose range tested in Phase 1 studies.

Platelet Function. In Study F1K-LC-GUAD, platelet function in response to arachidonic acid, adenosine diphosphate, and ristocetin was independent of infusion rate, infusion duration, and estrogen status. The lack of effect of drotrecogin alfa (activated) on platelet function was a consistent finding in all studies in which platelet function was assessed.

Factor V and Factor VIII. In Study F1K-LC-GUAD, Factor V levels decreased significantly during infusion in a manner dependent on infusion duration ($p=0.038$). Factor V levels did not depend on estrogen status.

Factor VIII levels declined during infusions. The decline depended on infusion duration ($p=0.010$), but not on estrogen status.

Prolonged administration of drotrecogin alfa (activated) was associated with a statistically significant decline in Factor V and, to a lesser extent, Factor VIII levels. However, both Factor V and Factor VIII levels remained within the normal range and this effect is probably of minor clinical significance.

Serum Electrolytes, Chemistry and Hematology Panels, and Urinalysis. Out-of-range levels from serum electrolyte panels, chemistry and hematology panels, and urinalysis panels were infrequent and appeared to be distributed randomly across the range of drotrecogin alfa (activated) infusion rates.

3.4. Safety Findings

Safety Findings in the Phase 1 Studies. In these studies, safety was assessed by analyzing adverse events, serious adverse events, laboratory data, and vital sign data. These analyses indicated no toxicities associated with the administration of drotrecogin alfa (activated) that precluded further clinical investigation of drotrecogin alfa (activated) as a therapeutic for patients with severe sepsis.

Mild headache and ecchymosis were the most frequent adverse events reported by subjects in the Phase 1 studies. The incidence of headache depended on infusion rate (dose), but not infusion duration. The etiology of headache is unclear. Most headache resolved with administration of acetaminophen. There was no neurological sequelae or need to discontinue drug as a result of headache. Ecchymosis was reported most often at the site of venipuncture. Additionally, there was a higher than expected occurrence of occult blood in the urine in healthy subjects. Neither of these phenomena were associated with infusion rates (dose) but both of these findings are very likely to be the result of the antithrombotic and profibrinolytic properties of drotrecogin alfa (activated).

In the Phase 1 studies, only 1 subject developed a problem that was reported as three serious adverse events (dysuria, hematuria, and neoplasm). The events occurred during the outpatient waiting period between drotrecogin alfa (activated) infusions (first infusion of 6 hours and second infusion of 24 hours). Subject 2041, a 72-year-old male, developed hematuria 8 days after completion of the 6-hour infusion. His coagulation parameters were normal when he was released to outpatient follow-up. The subject was re-admitted to the clinical pharmacology facility, which met the serious event criteria by extending the subject's hospitalization and making a diagnosis of cancer. He was found to have a filling defect in his right renal calyx. A follow-up cystoscopy revealed two small tumors in the bladder and a mass in the right renal calyx consistent with transitional cell carcinoma. The subject discontinued from the study and did not receive a second infusion. The subject was transferred into the care of an urologist at the Indiana University Hospitals and then to Veterans Administration for surgery and follow-up. The investigator judged these events to be a typical presentation for transitional cell carcinoma and unrelated to drotrecogin alfa (activated) administration.

Immunogenicity. See Section 10.

3.5. Conclusions

- In normal healthy subjects, the pharmacokinetics of drotrecogin alfa (activated) were linear through infusion rates of 48 μg/kg/hr. V_{ss} was small, and elimination was biphasic with a rapid initial phase ($t_{1/2\alpha}$ = 13 minutes) and a slower second phase ($t_{1/2\beta}$ = 1.6 hours). Because of these short half-lives, 90% of the steady-state plasma concentration is reached within 2 hours of starting a constant rate infusion, and 90% is eliminated within 2 hours of stopping the infusion.

- The time course of pharmacodynamic response to drotrecogin alfa (activated) in healthy subjects, expressed as change from baseline whole blood APTT (ΔAPTT), paralleled the time course of Activated Protein C concentration in plasma. The change from baseline whole blood prothrombin time (ΔPT) also increased with increasing plasma APC concentration, but the magnitude of change in ΔPT_{max} was very small. Factor V and Factor VIII levels decreased with increasing infusion duration. Estrogen status did not affect the relationship between APTT or PT and plasma APC concentration. There was no evidence of hysteresis in any of the pharmacodynamic parameters affected by APC.
- Mild headache and ecchymosis were the most frequent adverse events reported in healthy subjects.

These pharmacokinetic and safety profiles supported continued development of drotrecogin alfa (activated) as a potential therapy for the treatment of severe sepsis.

4. Dose Selection

The Phase 2 study F1K-MC-EVAA was a randomized, double-blind, placebo-controlled study designed to investigate the safety and pharmacokinetics of drotrecogin alfa (activated) in patients with severe sepsis and to determine an effective infusion rate and duration for use in subsequent studies. An effective infusion rate and infusion duration were determined by evaluating the effect of drotrecogin alfa (activated) administration on the coagulation abnormalities associated with severe sepsis. The primary markers of coagulation measured in the study were D-dimer level, fibrinogen level, and platelet count, and the primary marker of inflammation measured was IL-6. The protocol-specified primary analysis was based on the treatment to which the patient was randomly assigned irrespective of the actual dose received. Supplemental analyses were performed based on drug exposure (μg/kg) and the steady-state concentration achieved (ng/mL).

A statistically significant dose-dependent decline in D-dimer and IL-6 levels was observed in drotrecogin alfa (activated) patients compared with placebo patients. This dose-dependent effect was evident regardless of the type of analysis conducted (ie, by treatment assignment, by drug exposure, or by steady-state concentration achieved). An infusion rate of 24 μg/kg/hr for 96 hours was associated with the largest reduction in D-dimer and IL-6 levels. No safety concerns were identified that would limit the infusion rate or duration. This infusion rate and duration were recommended for use in the Phase 3 pivotal trial (Study F1K-MC-EVAD).

4.1. Overview

Eligible patients were male or female, 18 years or older with severe sepsis who met criteria for systemic inflammatory response syndrome and associated organ failure. Patients must have met inclusion criteria within a 24-hour time period. From the time a patient met inclusion criteria, an additional 36 hours were allowed for the investigator to obtain informed consent, complete randomization, and initiate the study drug infusion.

This study was conducted in two sequential steps designated as Stage 1 and Stage 2. Both Stage 1 and Stage 2 were randomized, double-blind, placebo-controlled, dose-ranging studies of drotrecogin alfa (activated) or placebo administered as a continuous intravenous infusion over a fixed interval of 48 hours (Stage 1) or 96 hours (Stage 2).

Stage 1. The safety, pharmacokinetics, and pharmacodynamics of a 48-hour infusion of drotrecogin alfa (activated) were evaluated using a dose-escalation scheme with an initial infusion rate of 12 μg/kg/hr. Patients were randomly assigned to drotrecogin alfa (activated) or placebo treatment in a 2:1 ratio. Five dosing groups were formed. After the first dosing group had received an infusion rate of 12 μg/kg/hr, an unblinded Data Monitoring Board was convened to review the available safety, pharmacokinetic, and pharmacodynamic data from this dosing group. Based on these data, the Data Monitoring Board determined the infusion rate to be received by the next dosing group.

Stage 2. A 96-hour infusion was evaluated using a dose-escalation scheme similar to that used in Stage 1. Patients were randomly assigned to drotrecogin alfa (activated) or placebo treatment in a 3:1 ratio. Four dosing groups were formed. The initial infusion rate was determined by the Data Monitoring Board, based on Stage 1 results. As in Stage 1, the unblinded Data Monitoring Board was convened to review the available safety, pharmacokinetic, and pharmacodynamic data from the first dosing group and to determine the infusion rate to be received by the next dosing group.

In both stages, bedside whole blood APTT testing was performed 4 hours after the initiation of the study drug infusion and every 24 hours for the duration of the infusion. The assay was performed within two minutes of the sample being obtained from the subject. Use of whole blood minimized the time necessary to prepare the sample for measurement of APTT and PT. Conduct of the assay at the bedside eliminated the time necessary to transport the sample to the hospital laboratory.

If a patient's bedside whole blood APTT was ≥95 seconds, then the patient's infusion rate was reduced by 25% of the original dose and whole blood APTT was retested in 4 hours. After retesting, if the whole blood APTT was <95 seconds, the current dose was maintained; if the whole blood APTT was ≥95 seconds, the dose was again reduced by 25% of the original dose. This process continued until the patient's whole blood APTT was <95 seconds. The decision to reduce the infusion rate based on the bedside whole blood APTT was prospectively defined and based on standard practices for heparin use.

Figure 4.1 shows the study design and treatment groups for Study F1K-MC-EVAA.

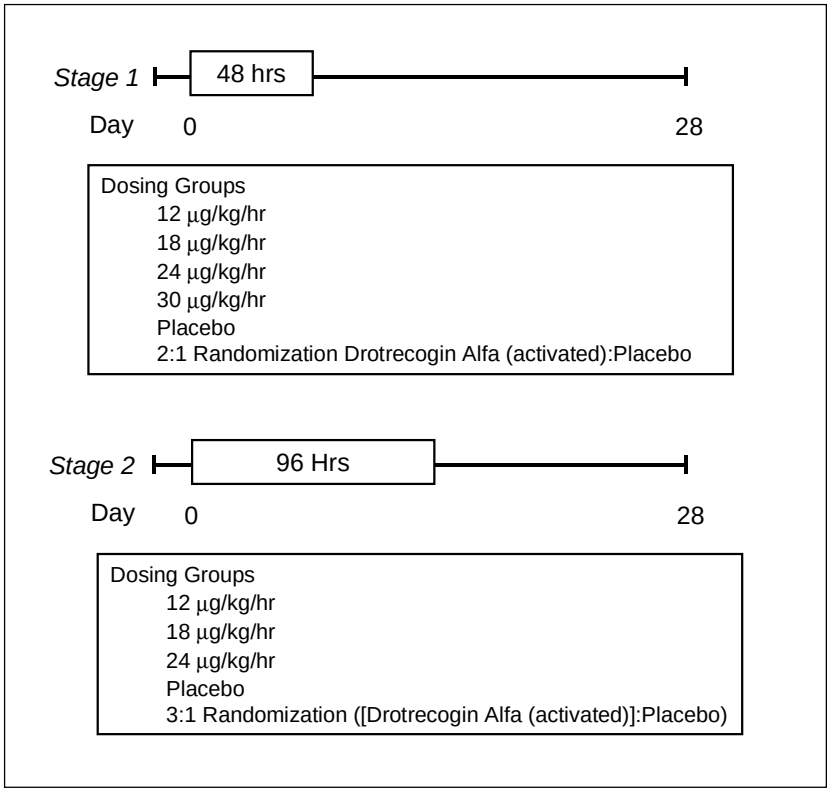


Figure 4.1. Study design and treatment group assignments for Study F1K-MC-EVAA.

Statistical Methods. The primary population of interest consisted of all enrolled patients who received study drug infusion [drotrecogin alfa (activated) or placebo] for any length of time. For analysis, patients were allocated to the treatment group to which they were randomly assigned.

D-dimer and IL-6 levels are presented as percent change from baseline summary statistics at each postbaseline time point. At each time point, analysis of variance (ANOVA) based on ranked data was used to draw statistical conclusions. In addition to the primary analyses, monotonic dose response analyses were performed using the percent change from baseline to end of infusion data for all patients. The last-observation-carried-forward (LOCF) method was chosen as the primary imputation method due to the impact of death and administrative sample handling resulting in missing data. Two-sided p-values ≤ 0.05 are noted as statistically significant with no adjustment for the multiple comparisons performed.

The impact of drotrecogin alfa (activated) administration on coagulation and inflammation markers was also assessed by allocating patients to treatment groups according to total drotrecogin alfa (activated) drug exposure. The drotrecogin alfa (activated) patient population was segmented into exposure quartiles within each study

stage. The total drug exposure for each patient was calculated as the sum of the dose received (μg/kg/hr) at every time point during infusion, thus yielding a total exposure expressed in μg/kg. Patients who died prior to the end of their assigned infusion period had the dose received at the time of death carried forward for the duration of their assigned infusion to eliminate the impact of death on the exposure calculation.

For each primary coagulation and inflammation marker, graphics portraying a correlation analysis of the percent change from baseline to end of infusion marker results with patient predicted APC steady-state concentrations are presented. A two-sided p-value based on a test for zero correlation using Spearman's rank correlation is calculated.

4.2. Results

4.2.1. Patient Disposition

In Study F1K-MC-EVAA, 135 patients were entered and randomly assigned to drotrecogin alfa (activated) or placebo treatment. Four patients withdrew from the study before receiving study drug: 3 patients did not meet entry criteria and 1 patient withdrew consent. Of the 131 patients who received study drug, 90 received drotrecogin alfa (activated) (Stage 1: 46 patients; Stage 2: 44 patients) and 41 received placebo (Stage 1: 26 patients; Stage 2: 15 patients).

Table 4.1 contains a summary of treatment assignments and the number of drotrecogin alfa (activated) patients who did not receive a full dose of study drug or received a change in dose. Patients were defined as receiving a full dose of study drug if they received a study drug infusion of at least 47 hours during Stage 1 or at least 95 hours during Stage 2 with no change in their initial infusion rate. Patients were defined as having a change in dose if their infusion rate was reduced or increased for dose error reasons, or was reduced because of an elevated bedside whole blood APTT.

Table 4.1. Summary of Patients Receiving a Full Dose of Study Drug or Requiring a Change in Dose Study F1K-MC-EVAA

Treatment Group	Number of Patients	Number of Patients Receiving a Full Dose n (%)	Number of Patients Requiring a Change in Dose n (%)
Drotrecogin Alfa (Activated)			
48-Hour Infusion			
12 µg/kg/hr	11	6 (54.5)	0
18 µg/kg/hr	11	7 (63.6)	0
24 µg/kg/hr	12	9 (75.0)	1 (8.3)
30 µg/kg/hr	12	5 (41.7)	6 (50.0)
96-Hour Infusion			
12 µg/kg/hr	14	8 (57.1)	1 (7.1)
18 µg/kg/hr	15	7 (46.7)	1 (6.7)
24 µg/kg/hr	15	5 (33.3)	7 (46.7)
All Drotrecogin Alfa (Activated)	90	47 (52.2)	16 (17.8)
Placebo	41	26 (63.4)	3 (7.3)

Abbreviations: n = number of patients.
Source: Table EVAA.11.14 and Table EVAA.11.16 (F1K-MC-EVAA Clinical Study Report)

Six of the 12 patients who received drotrecogin alfa (activated) 30 µg/kg/hr for 48 hours had a change in infusion rate because of a prolonged bedside whole blood APTT. Also, 6 patients in the 30 µg/kg/hr had maximum Activated Protein C steady-state concentrations greater than 250 ng/mL. Although there were no safety concerns noted in this treatment group, an infusion rate of 30 µg/kg/hr was not evaluated during Stage 2 (96-hour infusion) based on these results.

4.2.2. Patient Characteristics

Table 4.2 contains a summary of the demographic characteristics of patients who received study drug.

Table 4.2. Summary of Demographic Characteristics Study F1K-MC-EVAA

Variable	Drotrecogin Alfa (activated) N=90	Placebo N=41	Total N=131
Age			
Mean	58.5	62.2	59.7
Range	22.8–88.4	19.7–89.7	19.7–89.7
Age Category, n (%)			
<65	59 (65.6)	19 (46.3)	78 (59.5)
≥65	31 (34.4)	22 (53.7)	53 (40.5)
Gender, n (%)			
Male	57 (63.3)	27 (65.9)	84 (64.1)
Female	33 (36.7)	14 (34.1)	47 (35.9)
Racial Origin, n (%)			
Caucasian	71 (78.9)	35 (85.4)	106 (80.9)
Noncaucasian	19 (21.1)	6 (14.6)	25 (19.1)
Country, n (%)			
Canada	29 (32.2)	17 (41.5)	46 (35.1)
United States	61 (67.8)	24 (58.5)	85 (64.9)

Abbreviations: N = number of patients in the treatment group; n = number of patients within the treatment group who share the characteristic.
Source: PC001#1#, PC00313#, Table EVAA11.2 (F1K-MC-EVAA Clinical Study Report).

4.2.3. Analyses of Coagulation and Inflammation Markers by Treatment Assignment

The protocol-specified primary analysis was based on the treatment to which the patient was randomly assigned regardless of the actual dose received.

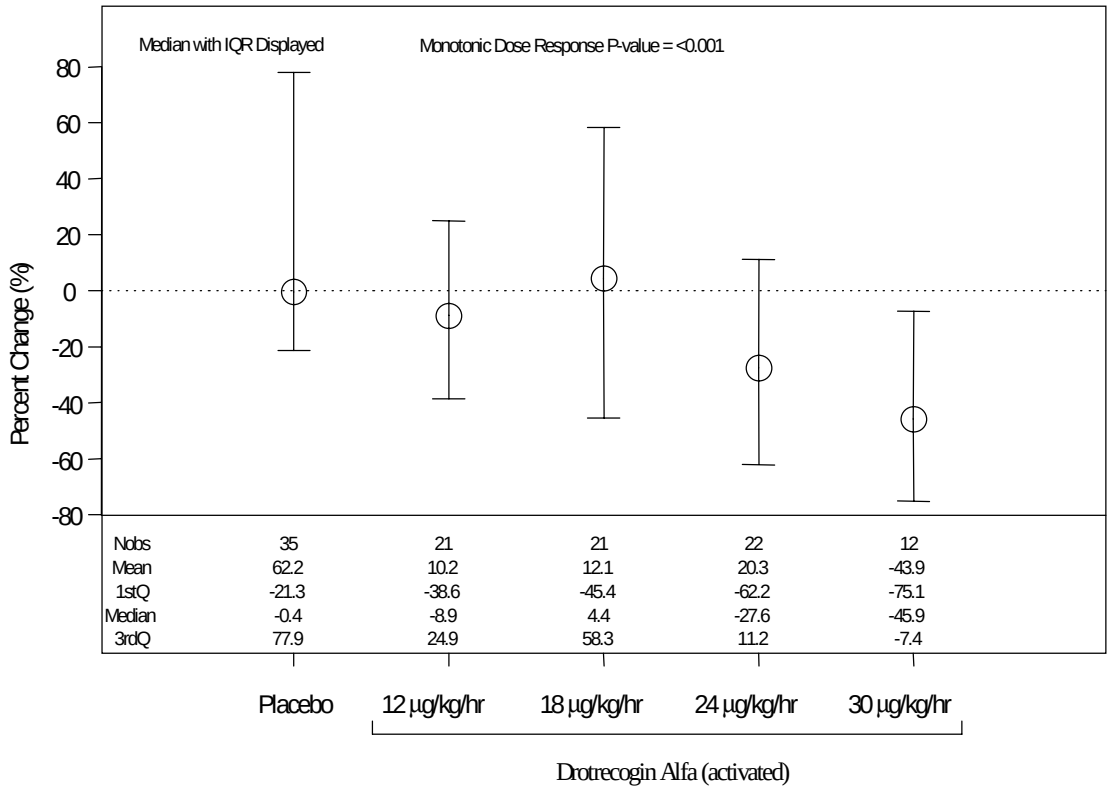
D-Dimer Analyses. D-dimer levels were analyzed at baseline and daily through Study Day 5 for Stage 1 patients or daily through Study Day 7 for Stage 2 patients. Stage 1 patients were assigned to the 12 μg/kg/hr, 18 μg/kg/hr, 24 μg/kg/hr, 30 μg/kg/hr, or placebo treatment group. Stage 2 patients were assigned to the 12 μg/kg/hr, 18 μg/kg/hr, 24 μg/kg/hr, or placebo treatment group.

For Stage 1 (48-hour infusion) patients, statistically significant differences in percent change from baseline D-dimer levels were observed among the five treatment groups at Study Days 2 through 5 (p=0.05, p=0.01, p=0.01, and p=0.05, respectively). The greatest percent decreases were observed in the 30 μg/kg/hr treatment group.

For Stage 2 (96-hour infusion) patients, statistically significant differences in percent change from baseline D-dimer levels were observed among the four treatment groups on

Study Days 2 through 4 (p=0.01, p=0.03, and p=0.02, respectively). The greatest percent decreases were observed in the 24 µg/kg/hr treatment group.

D-dimer Dose Response Analysis. Figure 4.2 shows the monotonic dose response with respect to the percent change from baseline D-dimer levels at the end of infusion (p<0.001). The drotrecogin alfa (activated) doses ranged from 12 µg/kg/hr to 30 µg/kg/hr with 48-hour and 96-hour infusion durations combined.



End of Infusion is Study Day 2 for Stage 1 Patients and Study Day 4 for Stage 2 Patients
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Source is EVAA Final Report Graphs. SSC. Data from RMP.SAS.F1KM.MCEVAASC.FINAL.
Data analyzed using analysis of variance (ANOVA) on the ranks. Missing data imputation method:LOCF.

Figure 4.2. Percent change from baseline D-dimer levels at the end of the infusion by dose for all patients. Study F1K-MC-EVAA.

Fibrinogen and Platelet Analyses. Fibrinogen and platelet levels were analyzed at baseline and daily through Study Day 5 for Stage 1 patients or daily through Study Day 7 for Stage 2 patients. No statistically significant dose-dependent effects of drotrecogin

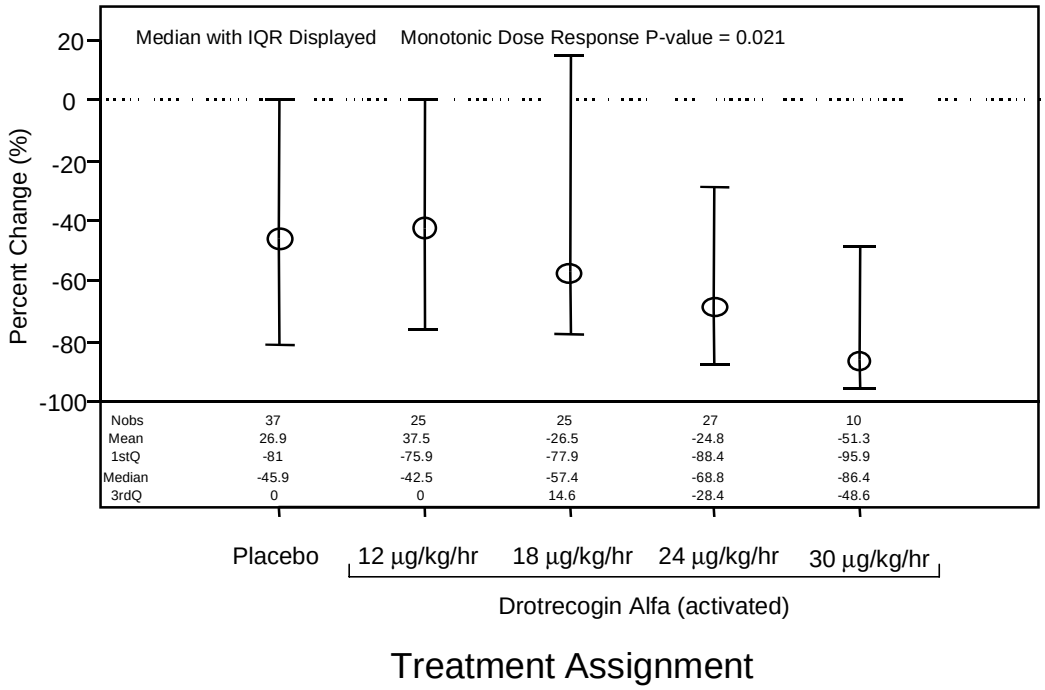
alfa (activated) administration on changes in fibrinogen concentrations or platelet counts were demonstrated in this study. The observation that 76 out of 104 patients (73.1%) with available baseline data had fibrinogen concentrations above the upper limit of normal suggests that fibrinogen concentrations are more predictive of an acute phase reaction rather than disseminated intravascular coagulation. Likewise, of the patients with available baseline platelet count data, 70 out of 104 patients (67.3%) had platelet counts within the normal range, approximately 20% of patients had thrombocytopenia, and the remaining patients exhibited thrombocytosis (acute phase response), suggesting that platelet count was not an early predictor of the sepsis-associated coagulopathy.

IL-6 Analyses. IL-6 levels were analyzed at baseline and daily through Study Day 3 for Stage 1 patients or daily through Study Day 5 for Stage 2 patients.

For Stage 1 (48-hour infusion) patients, statistically significant differences between the five treatment groups in percent change from baseline were observed on Study Days 1 and 3 ($p=0.04$ and $p=0.02$, respectively). The greatest percent decreases from baseline were observed in the 30 μg/kg/hr treatment group.

For Stage 2 (96-hour infusion) patients, no statistically significant differences between the four treatment groups in percent change from baseline IL-6 levels were observed. Although the differences between the treatment groups were not statistically significant, the greatest percent decreases were observed in the 24 μg/kg/hr treatment group.

IL-6 Dose Response Analysis. Figure 4.3 shows the monotonic dose response with respect to the percent change from baseline IL-6 levels at the end of infusion ($p=0.021$). The drotrecogin alfa (activated) doses ranged from 12 μg/kg/hr to 30 μg/kg/hr with 48-hour and 96-hour infusion durations combined.



End of Infusion is Study Day 2 for Stage 1 Patients and Study Day 4 for Stage 2 Patients
F1K-MC-EVAA Final Report. Report Produced Fri Mar 3 11:34:49 2000
Source is EVAA Final Report Graphs.$_{ssc}$. Data from RMP.SAS.F1KM.MCEVAASC.FINAL.
Data analyzed using analysis of variance (ANOVA) on the ranks. Missing data imputation method:LOCF.

Figure 4.3. **Percent change from baseline in IL-6 levels at the end of the infusion by dose received. Study F1K-MC-EVAA.**

4.2.4. Analyses of Coagulation and Inflammation Markers by Drug Exposure

The impact of drotrecogin alfa (activated) on coagulation and inflammation markers was assessed by allocating patients to treatment groups according to total drotrecogin alfa (activated) received. The drotrecogin alfa (activated) patient population was segmented into exposure quartiles within each study stage. Table 4.3 contains the total drug exposure quartiles to which drotrecogin alfa (activated) patients were allocated.

Table 4.3. Standardized Total Drug Exposure Data Summary by Quartile[a] Study F1K-MC-EVAA

	Quartile			
Infusion Rate and Duration	1st	2nd	3rd	4th
48-hour Infusion				
Number of Patients	11	12	14	9
Mean (μg/kg/hr)	11.0	16.3	22.3	28.1
Range (μg/kg/hr)	(8.3–12.0)	(12.0–18.0)	(18.0–24.0)	(24.0–30.2)
96-hour Infusion				
Number of Patients	10	12	11	11
Mean (μg/kg/hr)	6.8	12.4	17.4	21.1
Range (μg/kg/hr)	(4.3–11.4)	(12.0–15.2)	(15.4–18.0)	(18.0–24.3)

[a] Standardized total drug exposures (in μg/kg/hr) were calculated as total drug exposure (in μg/kg) divided by 48 hours for Stage 1 patients and total drug exposure (in μg/kg) divided by 96 hours for Stage 2 patients.

Source: Table EVAA.11.1 (F1K-MC-EVAA Clinical Study Report).

D-dimer Analyses. A statistically significant difference in percent change from baseline D-dimer levels during Stage 1 (48-hour infusion) was observed among the five treatment groups at Study Days 2 through 5 (p=0.02, p=0.005, p=0.01, and p=0.04). The most favorable clinical and statistical responses in the percent change from baseline D-dimer levels over time were observed in the third and fourth drotrecogin alfa (activated) exposure quartiles, which corresponded to mean standardized total drug exposures of 22.3 μg/kg/hr and 28.1 μg/kg/hr, respectively.

No statistically significant differences in percent change from baseline D-dimer levels during Stage 2 (96-hour infusion) was observed among the five treatment groups. However, a statistically significant difference was observed between placebo patients and drotrecogin alfa (activated) patients in the fourth exposure quartile on Study Days 3, 4, and 5 (pairwise p=0.04, p=0.02, and p=0.04). The fourth exposure quartile in the 96-hour infusion duration corresponded to a mean standardized total drug exposure dose of 21.1 μg/kg/hr.

Fibrinogen and Platelet Analyses. During Stage 1 (48-hour infusion), no statistically significant differences in percent change from baseline fibrinogen level or platelet count by exposure quartile were observed among the five treatment groups.

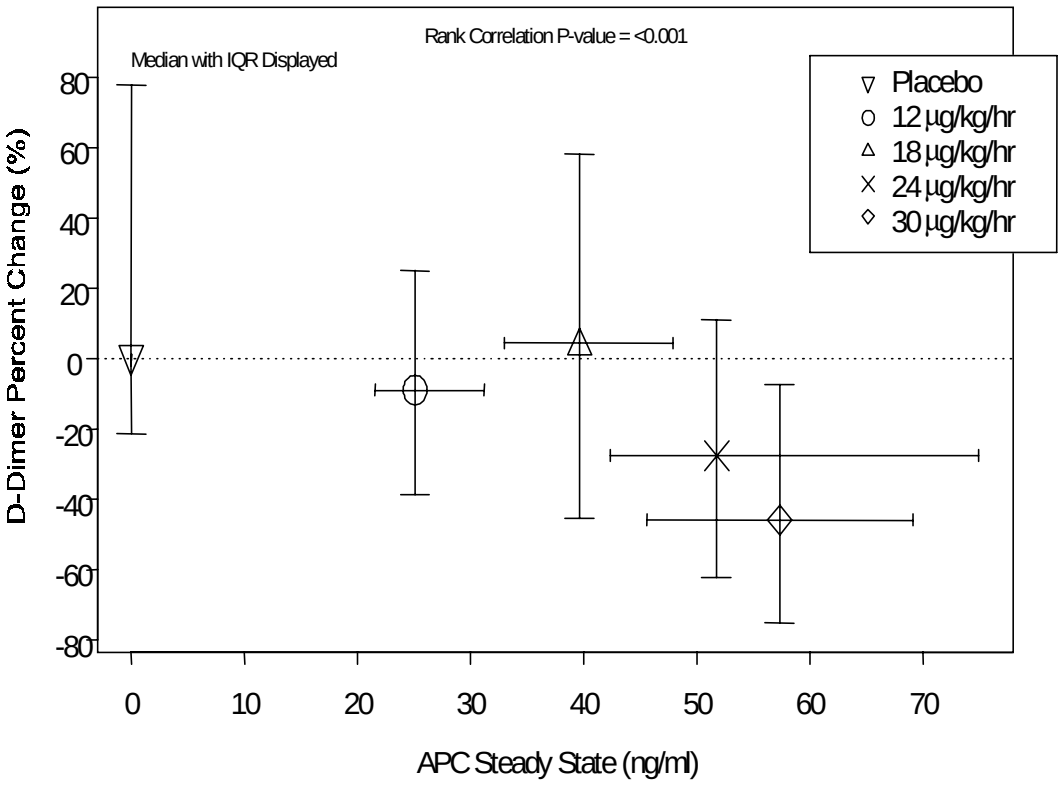
During Stage 2 (96-hour infusion), no statistically significant differences in percent change from baseline fibrinogen level by exposure quartile were observed among the five treatment groups. However, a statistically significant difference in median percent change from baseline platelet count was observed on Study Day 4 ($p=0.01$).

IL-6 Analyses. During Stage 1 (48-hour infusion), a statistically significant difference in percent change from baseline-to-end-of-infusion IL-6 levels by exposure quartile was observed among the five treatment groups at Study Days 1, 2, and 3 ($p=0.03$, $p=0.02$, and $p=0.02$, respectively). The greatest percent decreases were observed in the fourth exposure quartile.

During Stage 2 (96-hour infusion), no statistically significant differences in percent change from baseline-to-end-of-infusion IL-6 levels by exposure quartile were observed among the five treatment groups.

4.2.5. Analyses of Coagulation and Inflammation Markers by Steady-State Concentration Achieved

D-Dimer Analyses. A correlation analysis of the percent change from baseline-to-end-of-infusion D-dimer measurements with patients' steady-state APC concentrations is presented in Figure 4.4. Based on a test for zero correlation using Spearman's rank correlation, a statistically significant positive correlation was observed between predicted APC steady-state concentration and percent decrease from baseline D-dimer levels at the end of infusion ($p<0.001$).



EOI LOCF data used for PD marker, Time averaged APC steady state concentrations used as PK response

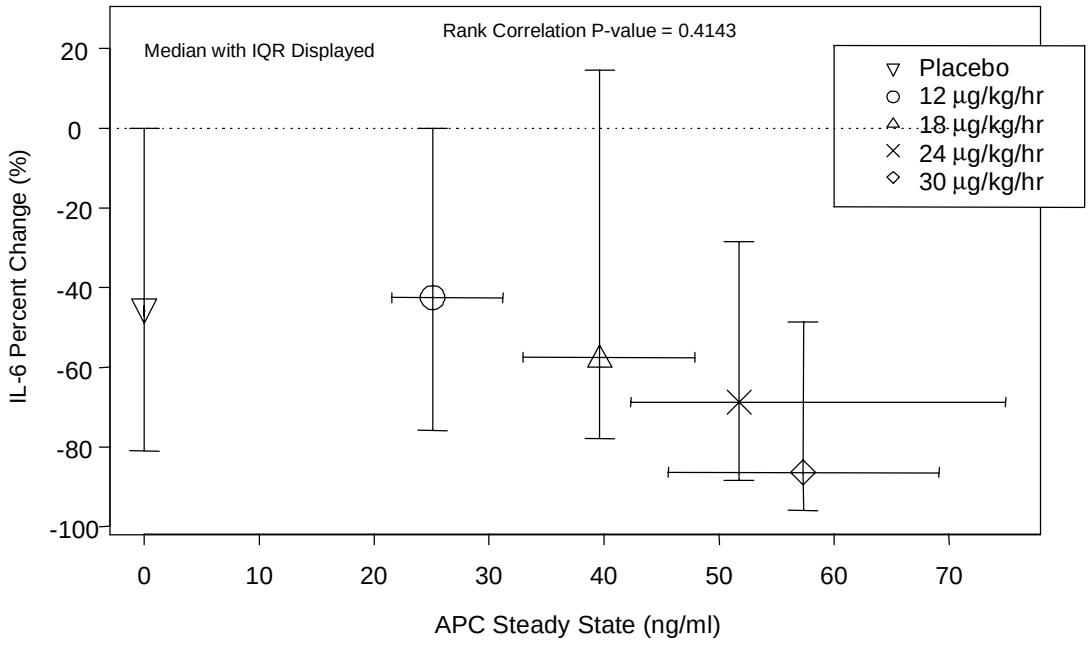
Figure 4.4. **APC steady-state concentration versus percent change at end-of-infusion D-dimer levels for all patients. Study F1K-MC-EVAA.**

Median percent change from baseline D-dimer values and interquartile range are displayed with median APC steady-state concentration and interquartile range.

Fibrinogen and Platelet Analyses. A correlation analysis of percent change from baseline to end of infusion fibrinogen and platelet measurements with patients' APC steady-state concentrations was performed. Based on a test for zero correlation using Spearman's rank correlation, the correlation between predicted APC steady-state concentration and percent decrease from baseline fibrinogen and platelet levels at the end of infusion were not statistically significant.

IL-6 Analyses. A correlation analysis of percent change from baseline to end of infusion IL-6 measurements with patients' APC steady-state concentrations is presented in Figure 4.5. Based on a test for zero correlation using Spearman's rank correlation, the

correlation between predicted APC steady-state concentrations and percent decrease from baseline IL-6 levels at the end of infusion was not statistically significant.



EOI LOCF data used for PD marker, Time averaged APC steady state concentrations used as PK response

Figure 4.5. **APC steady-state concentration versus percent change from baseline at end-of-infusion IL-6 levels for all patients. Study F1K-MC-EVAA.**

Median percent change from baseline IL-6 values and interquartile range are displayed with median APC steady-state concentration and interquartile range.

4.2.6. Safety Analyses

28-Day All-Cause Mortality. In consideration of the trial size, it was not anticipated a-priori that statistically significant treatment effects with respect to 28-day all cause mortality would be detected and, in fact, no statistically significant differences were demonstrated.

Table 4.4 contains a summary of the number of deaths within 28 days (672 hours) after the start of study drug infusion for each treatment group. None of the deaths were considered by the site investigator to be related to study drug.

When comparing all drotrecogin alfa (activated) with all placebo patients, a 15% relative risk reduction in 28-day all-cause mortality was observed (28.9% versus 34.1%; p=0.545).

Table 4.4. 28-Day All-Cause Mortality By Treatment Group Study F1K-MC-EVAA

Treatment Group	Number of Patients	Survived n (%)	Died n (%)	p-Value[a]
Drotrecogin Alfa (Activated)				
48-Hour Infusion Duration				
12 µg/kg/hr	11	8 (72.7)	3 (27.3)	0.386[b]
18 µg/kg/hr	11	8 (72.7)	3 (27.3)	
24 µg/kg/hr	12	12 (100)	0	
30 µg/kg/hr	12	9 (75.0)	3 (25.0)	
96-Hour Infusion Duration				
12 µg/kg/hr	14	9 (64.3)	5 (35.7)	
18 µg/kg/hr	15	8 (53.3)	7 (46.7)	
24 µg/kg/hr	15	10 (66.7)	5 (33.3)	
All Drotrecogin Alfa (Activated) Patients	90	64 (71.1)	26 (28.9)	0.545[c]
Placebo				
48-Hour Infusion	26	16 (61.5)	10 (38.5)	
96-Hour Infusion	15	11 (73.3)	4 (26.7)	
All Placebo Patients	41	27 (65.9)	14 (34.1)	

Abbreviation: n = number of patients.

[a] Two-sided p-values from Pearson's chi-square.

[b] P-value for comparison of all treatment groups.

[c] P-value for comparison of all drotrecogin alfa (activated) patients and all placebo patients.

Source: MS001#1#, MS002#5#, MS00213# (F1K-MC-EVAA Clinical Study Report).

Figure 4.6 presents the Kaplan-Meier survivals curves for drotrecogin alfa (activated) and placebo patients.

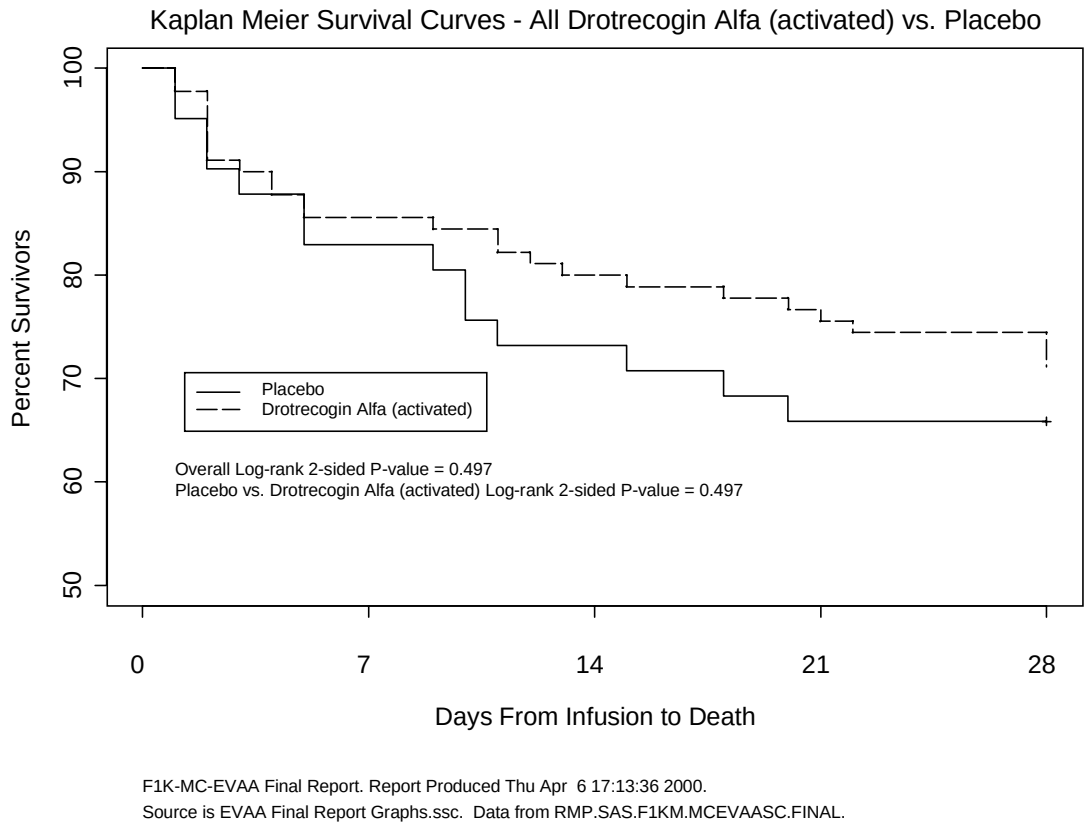


Figure 4.6. **Kaplan-Meier survival curves for all drotrecogin alfa (activated)-treated and placebo-treated patients. Study F1K-MC-EVAA.**

Adverse Event Analyses. Table 4.5 contains a summary of adverse events that occurred in drotrecogin alfa (activated) treated patients by infusion duration (48 hours or 96 hours). There were no statistically significant differences in the percentage of patients who experienced a treatment-emergent adverse event or serious adverse event between 48-hour-infusion dosing groups and 96-hour-infusion dosing groups. The number of patients who experienced a bleeding event reported as a serious adverse event during the study drug infusion period was small in both infusion duration dosing groups: 2 patients who received a 48-hour infusion and 1 patient who received a 96-hour infusion. In the final analysis of bedside whole blood APTT levels observed in this study, there was no apparent correlation between bedside whole blood APTT levels and the risk of bleeding.

Table 4.5. Brief Summary of Adverse Events by Infusion Duration Study F1K-MC-EVAA

Adverse Event Study Period	Drotrecogin Alfa (Activated) 48-Hour Infusion N = 46	 96-Hour Infusion N = 44	All Placebo N = 41	p-Value[a]
Treatment-Emergent Adverse Events				
Infusion Period	28 (60.9%)	28 (63.6%)	24 (58.5%)	0.890
28-Day Study Period	36 (78.3%)	30 (68.2%)	30 (73.2%)	0.558
Serious Adverse Events				
Infusion Period	7 (15.2%)	12 (27.3%)	10 (24.4%)	0.355
28-Day Study Period	15 (32.6%)	20 (45.5%)	19 (46.3%)	0.337
Bleeding Events Reported as Serious Adverse Events				
Infusion Period	2 (4.3%)	1 (2.2%)	0	—
28-Day Study Period	3 (6.5%)	1 (2.2%)	2 (4.9%)	—

Abbreviation: N = number of patients.

[a] Two-sided p-values from chi-square test for equality.

Source: Tables EVAA.12.1 (AE019#1#, AE020#2#, AE021#3#), EVAA.12.2 (AE001#1#, AE002#2#, AE003#3#), EVAA.12.11 (AE030#1#, AE03313#, AE031#3#), EVAD.12.12 (AE009#1#, AE011#3#, AE01213#), EVAA.12.7 (PC015#5#, PS001#5#, BD017#5#), EVAA.12.9 (PD004#5#).

4.3. Assessment of Infusion Rate and Infusion Duration

As assessed by infusion rate, the maximum observed pharmacodynamic effect of drotrecogin alfa (activated) on D-dimer and IL-6 levels was observed at infusion rates of 24 μg/kg/hr and 30 μg/kg/hr. The infusion rate of 30 μg/kg/hr was not studied for longer than a 48-hour infusion duration because of the large number of patients having their infusion rate decreased due to a protocol-specified safeguard based on bedside whole blood APTT measurements. Although limited by the number of patients enrolled in the study, there was no apparent association between the rate of drotrecogin alfa (activated) infusion and the incidence of treatment-emergent adverse events, serious adverse events, bleeding events, or deaths thought to be related to study drug.

As assessed by infusion duration, the maximum observed pharmacodynamic effect of drotrecogin alfa (activated) on D-dimer levels occurred at the end of 96 hours of infusion for the 24 μg/kg/hr treatment group. In addition, although limited by the number of patients enrolled in the study, there was no apparent association between the duration of drotrecogin alfa (activated) infusion and the incidence of treatment-emergent adverse events, serious adverse events, bleeding events, or deaths thought to be related to study drug.

Similar findings were observed when the data were analyzed by total drug exposure. The maximum D-dimer and IL-6 concentration reductions over time were observed in the third and fourth drotrecogin alfa (activated) exposure quartiles for the 48-hour infusion duration, corresponding to mean standardized total drug exposures of 22.3 μg/kg/hr and 28.1 μg/kg/hr drotrecogin alfa (activated). Additionally, the maximum D-dimer concentration reduction over time was observed in the fourth exposure quartile, corresponding to a mean standardized total drug exposure infusion rate of 21.1 μg/kg/hr for the 96-hour infusion duration.

Based on these results, the infusion rate of 24 μg/kg/hr for 96 hours was recommended for use in subsequent studies.

5. Efficacy

5.1. Overview of Study F1K-MC-EVAD

Based on overall pharmacodynamic and safety findings of Study F1K-MC-EVAA, the dose of 24 μg/kg/hr for 96 hours of continuous intravenous infusion was selected for use in an adequately powered (with respect to 28-day all-cause mortality), randomized, double-blind, placebo-controlled, multicenter Phase 3 study. The primary objective of the Phase 3 study F1K-MC-EVAD was to demonstrate that drotrecogin alfa (activated) reduced 28-day all-cause mortality in patients with severe sepsis.

5.1.1. Conduct of the Study

Eligible patients were at least 18 years old and had a diagnosis of severe sepsis, which was defined as meeting three or more SIRS criteria, having at least one sepsis-induced organ failure, and having a suspected or proven infection (see the publication based on this study in Appendix 5 for a summary of inclusion and exclusion criteria). Patients were randomly assigned to drotrecogin alfa (activated) or placebo treatment in a 1:1 ratio. All patients were followed to determine 28-day survival status.

Figure 5.1 shows the study design of Study F1K-MC-EVAD.

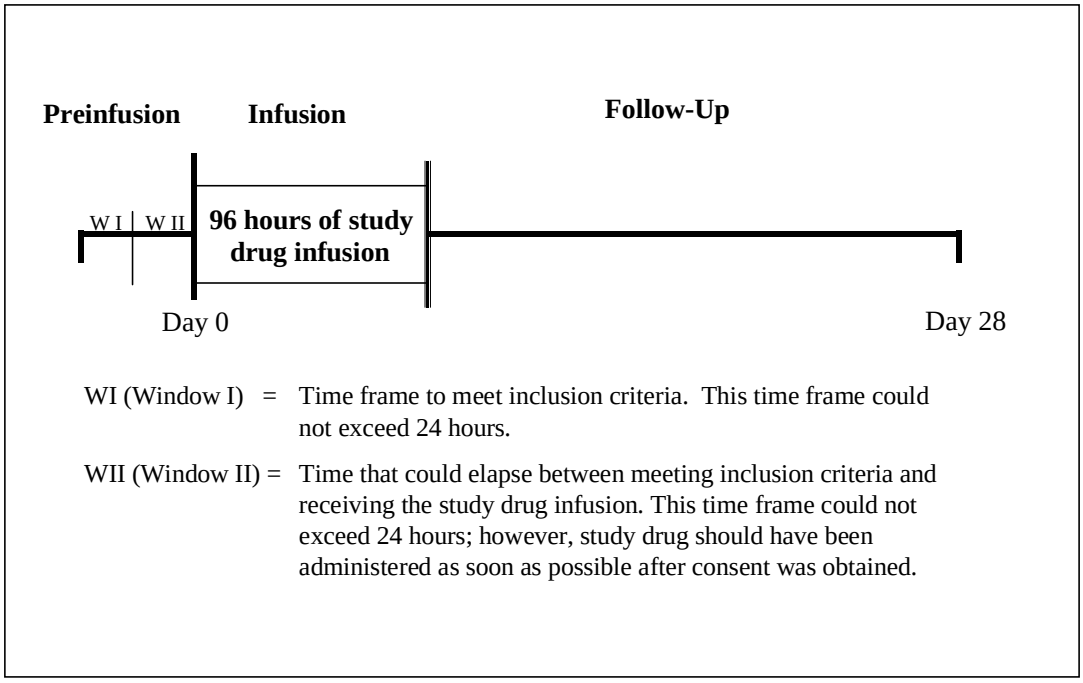


Figure 5.1. Design for Study F1K-MC-EVAD.

Approximately 2280 patients were to be enrolled in the study. Assuming that the true placebo 28-day all-cause mortality rate in the primary analysis population was 30%, this planned sample size was sufficient to ensure greater than 80% power to conclude efficacy if drotrecogin alfa (activated), in truth, was associated with an 18% relative risk reduction in 28-day all-cause mortality.

The primary efficacy endpoint was 28-day all-cause mortality. Patients were classified as "alive at Day 28" or, if they had died, "dead at Day 28" irrespective of the cause of death. The 28-day time point was defined to occur at exactly 672 hours from initiation of study drug infusion.

The prospectively defined primary analysis population included all patients who received study drug for any length of time. The prospectively defined primary analysis quoted p-value was based on a Cochran-Mantel-Haenszel test stratified by preinfusion APACHE II (Acute Physiology and Chronic Health Evaluation II) quartile (3 to 19, 20 to 24, 25 to 29, 30 to 53, respectively), age class (<60 or ≥60 years), and baseline Protein C activity class (≤40%, 41% to 60%, 61% to 80%, >80%, or unknown) for the primary analysis population (with pooling of underrepresented strata based on a prospectively defined pooling algorithm). The prospectively defined primary quoted relative risk and odds ratio and corresponding confidence intervals were calculated using the logit adjusted relative risk and odds ratio methods with an adjustment for preinfusion APACHE II quartile, age class, and baseline Protein C activity class (with pooling of underrepresented strata). Primary inference was based on the Cochran-Mantel-Haenszel test p-value.

5.1.2. Interim Analyses

Two interim analyses were planned and conducted under the auspices of an independent, external Data and Safety Monitoring Board: the first after 760 patients had completed the study (one-third of the planned study size) and the second after 1520 patients had completed the study (two-thirds of the planned study size). The Data and Safety Monitoring Board was composed of one statistician and three physicians with expertise in critical care. At each analysis, the Data and Safety Monitoring Board reviewed accumulating trial data and provided recommendations, based on prospectively defined guidelines, as to whether or not to continue patient enrollment in the study. The statistical guidelines to suspend trial enrollment for potential efficacy followed the O'Brien-Fleming method as implemented by Lan and Demets (O'Brien and Fleming 1979; Lan and Demets 1983). The two-sided critical alpha levels that were considered at the first and second interim analyses were 0.0002 and 0.0118, respectively. Additionally, if the predictive probability of success was low, trial enrollment was to be suspended.

5.1.3 Blinding

In order to maintain the integrity of the data and to ascertain that at no point information could be generated that could potentially unblind Lilly to the therapy assignment of any enrolled patient, the following processes were established for data flow and management:

- The patient treatment assignments were provided to investigators by Covance, a contract research organization, via a central randomization center. The code for the treatment assignment was retained by Covance.
- Data management was performed at Lilly without access to patient treatment assignments.
- Pharmacokinetic concentration measurements were performed by Lilly employees on patient samples with patient number obscured (by Covance). The results were sent to Covance for reconciliation of pharmacokinetic data with patient number.
- All deaths and serious adverse events were reviewed by Lilly in a blinded manner. If a death or serious adverse event was unexpected and considered drug related by the investigator, then a designated member of the pharmacovigilance group was able to unblind the patient's therapy by calling the randomization center. This form of unblinding was necessary to protect the safety of the patients and to satisfy the regulatory requirements.
- Lilly created the interim analysis datasets that were transferred to an external statistical service organization, Pat O'Meara Associates. The statistical service organization received the patient therapy assignments from Covance, performed the statistical analyses, generated the interim reports, and presented them to the Data and Safety Monitoring Board.

5.2. Patient Enrollment and Disposition

Table 5.1 contains a timeline of Study F1K-MC-EVAD showing the dates of approval and enrollment under the original and amended protocols, the timing of the interim analyses, and the stopping of the study.

Table 5.1. Timeline of Study F1K-MC-EVAD

Date	Event
28 July 1998	First patient enrolled under Protocol F1K-MC-EVAD
5 March 1999	Approval of amended Protocol F1K-MC-EVAD(a)
6 June 1999	First patient enrolled under amended Protocol F1K-MC-EVAD(a)
15 July 1999	Last patient enrolled under Protocol F1K-MC-EVAD
25 September 1999	External statistical services organization received trial data from Lilly and unblinded treatment codes from Covance
8 October 1999	First interim analysis conducted by independent Data and Safety Monitoring Board. Recommendation from Data and Safety Monitoring Board: "Continue the trial."
28 June 2000	Second interim analysis conducted by independent Data and Safety Monitoring Board. Recommendation from Data and Safety Monitoring Board: "Stop trial for highly statistically significant results."
26 July 2000	The last patient enrolled in the study completed the trial
27 July 2000	First unblinding of the interim trial results to a small component of the Lilly medical team
26 January 2001	Biologics License Application filed with Center for Biologics Evaluation and Research (CBER)

One protocol amendment, Amendment F1K-MC-EVAD(a), was approved on 5 March 1999. The amendment was approved six months prior to the first unblinding of the external statistical services organization statistician, who prepared analyses for the prospectively defined first interim analysis. The objectives of the amendment included the following:

1. Simplify the primary objective to clarify that there would be a single primary analysis that included all patients meeting the diagnosis of severe sepsis. To this end, references to the Protein C deficient subpopulation and the shock subpopulation were removed.
2. Clarify exclusion criteria for patients with esophageal varicies.
3. Add exclusion criteria for patients having undergone bone marrow, lung, liver, pancreas, or small bowel transplantation.
4. Add exclusion criteria for patients who were considered moribund and where death was imminent (within 24 hours).
5. Add exclusion criteria for patients whose family had not committed to aggressive management of the patient.
6. Add exclusion criteria for patients with acute pancreatitis without known infection.

7. Clarify exclusion criteria for patients with a history of malignancy.
8. Add exclusion criteria for patients having organ failure for greater than 24 hours at the time of meeting all inclusion criteria.
9. Change placebo from normal saline to 0.1% human serum albumin.
10. Replace "septic shock status" with "Protein C activity class" as a covariate for the primary analysis.

The first interim analysis occurred on 8 October 1999 and included a review of 760 patients who had received drotrecogin alfa (activated) or placebo. For the first interim analysis, the statistical guideline for stopping the study for significant efficacy was a two-sided p-value for the primary stratified 28-day all-cause mortality analysis of ≤0.0002 in favor of drotrecogin alfa (activated) treatment. After reviewing the trial data, the Data and Safety Monitoring Board recommended that trial enrollment continue with no changes in the conduct of the study.

The second interim analysis occurred on 28 June 2000 and included a review of 1520 patients who had received drotrecogin alfa (activated) or placebo. For the second interim analysis, the statistical guideline for stopping the study for significant efficacy was a two-sided p-value for both the prospectively defined primary stratified 28-day all-cause mortality analysis and the nonstratified analysis of ≤0.0118 for each analysis in favor of drotrecogin alfa (activated) treatment. After reviewing the trial data, the Data and Safety Monitoring Board recommended that study enrollment be suspended because of a highly statistically significant reduction in 28-day all-cause mortality in drotrecogin alfa (activated) patients compared with placebo patients. The study was halted after the second interim analysis because the positive efficacy signal and safety profile exceeded predetermined criteria. At the second interim analysis, 192 (25.0%) of the 768 drotrecogin alfa (activated) patients and 236 (31.38%) of the 752 placebo patients did not survive 28 days (primary stratified analysis p=0.0071, nonstratified p=0.0057).

Trial enrollment was suspended on 28 June 2000. At the time enrollment was suspended, 1728 patients had been enrolled in the trial, 1690 of these patients had received drotrecogin alfa (activated) or placebo for some length of time and constituted the prospectively defined primary analysis population for the study.

5.2.1. Patient Disposition

Of the 1728 enrolled patients, 38 discontinued from the study before receiving study drug: 21 of these patients were assigned to drotrecogin alfa (activated) treatment and 17 to placebo treatment. The primary reasons for discontinuation for these patients were: entry criteria not met (28 patients), physician decision (6 patients), sponsor decision (3 patients), and personal conflict or other patient decision (1 patient). All enrolled patients were followed for the entire 28-day period except for 1 patient randomized to the

drotrecogin alfa (activated) group. This patient did not receive study drug. This patient was classified as dead on Day 28 for analysis purposes.

Of the 1690 patients who received study drug (primary analysis population), 850 were randomly assigned to drotrecogin alfa (activated) treatment and 840 to placebo treatment.

5.2.2. Patient Characteristics

Table 5.2 contains a summary of the demographic characteristics and Table 5.3 contains a summary of illness characteristics of patients in the primary analysis population. At baseline, the demographic and disease severity characteristics were similar in the placebo treatment group and the drotrecogin alfa (activated) treatment group.

Patients enrolled into Study F1K-MC-EVAD met the clinical diagnosis of severe sepsis, defined as meeting three or more SIRS criteria, having at least one sepsis-induced organ failure, and having a suspected or proven infection (Appendix 5). Approximately 54% of the patients in the primary analysis population had the lung identified as the primary site of infection and approximately 20% had an intra-abdominal site of infection. The percentage of patients with pure gram-negative (22.5%) and pure gram-positive (25.4%) infections was similar. Approximately 75% of the patients had two or more sepsis-induced organ failures at study entry.

Table 5.2. Demographic Characteristics Study F1K-MC-EVAD

Variable	Drotrecogin Alfa (Activated) N=850 n (%)	Placebo N=840 n (%)	Total N=1690 n (%)
Gender			
Female	373 (43.9)	353 (42.0)	726 (43.0)
Male	477 (56.1)	487 (58.0)	964 (57.0)
Age			
Mean	60.46	60.61	60.53
Range	18.24–94.46	18.03–96.24	18.03–96.24
Age Classification			
<60	375 (44.1)	366 (43.6)	741 (43.8)
≥60	475 (55.9)	474 (56.4)	949 (56.2)
<65	437 (51.4)	449 (53.5)	886 (52.4)
≥65	413 (48.6)	391 (46.5)	804 (47.6)
<75	645 (75.9)	659 (78.5)	1304 (77.2)
≥75	205 (24.1)	181 (21.5)	386 (22.8)
Racial Origin			
African Descent	70 (8.2)	61 (7.3)	131 (7.8)
Western Asian	5 (0.6)	6 (0.7)	11 (0.7)
Caucasian	695 (81.8)	689 (82.0)	1384 (81.9)
East/SE Asian	9 (1.1)	13 (1.5)	22 (1.3)
Hispanic	34 (4.0)	40 (4.8)	74 (4.4)
Other	37 (4.4)	31 (3.7)	68 (4.0)
Country			
Australia	68 (8.0)	68 (8.1)	136 (8.0)
Belgium	76 (8.9)	73 (8.7)	149 (8.8)
Brazil	4 (0.5)	4 (0.5)	8 (0.5)
Canada	110 (12.9)	109 (13.0)	219 (13.0)
Germany	33 (3.9)	34 (4.0)	67 (4.0)
Spain	68 (8.0)	68 (8.1)	136 (8.0)
France	40 (4.7)	35 (4.2)	75 (4.4)
Netherlands	39 (4.6)	41 (4.9)	80 (4.7)
New Zealand	36 (4.2)	35 (4.2)	71 (4.2)
United States	352 (41.4)	353 (42.0)	705 (41.7)
South Africa	24 (2.8)	20 (2.4)	44 (2.6)

Abbreviations: N = number of patients in treatment group; n = number of patients within a treatment group had the characteristic; SE = Southeast.

Source: PC101#1# (F1K-MC-EVAD Clinical Study Report).

Table 5.3. Illness Characteristics
Study F1K-MC-EVAD

Variable	Drotrecogin Alfa (Activated) N=850	Placebo N=840	Total N=1690
Number of Organ Failure Entry Criteria Met, n (%)			
Zero	1 (0.1)	0	1 (0.1)
One	215 (25.3)	203 (24.2)	418 (24.7)
Two	270 (31.8)	273 (32.5)	543 (32.1)
Three	214 (25.2)	218 (26.0)	432 (25.6)
Four	119 (14.0)	116 (13.8)	235 (13.9)
Five	31 (3.6)	30 (3.6)	61 (3.6)
Shock and Ventilation Status, n (%)			
Shock Status (Yes response)	598 (70.4)	602 (71.7)	1200 (71.0)
Ventilation Status (Yes response)	623 (73.3)	652 (77.6)	1275 (75.4)
APACHE II Score			
1st APACHE II Quartile (3 to 19)	218 (25.6)	215 (25.6)	433 (25.6)
2nd APACHE II Quartile (20 to 24)	218 (25.6)	222 (26.4)	440 (26.0)
3rd APACHE II Quartile (25 to 29)	204 (24.0)	162 (19.3)	366 (21.7)
4th APACHE II Quartile (30 to 53)	210 (24.7)	241 (28.7)	451 (26.7)
Mean APACHE II Score	24.58	24.95	24.77
Site of Infection, n (%)			
Blood	45 (5.3)	42 (5.0)	87 (5.1)
Bone/Joint	3 (0.4)	8 (1.0)	11 (0.7)
Cardiac	6 (0.7)	3 (0.4)	9 (0.5)
CNS	20 (2.4)	19 (2.3)	39 (2.3)
Gynecologic	4 (0.5)	4 (0.5)	8 (0.5)
Head/EENT	4 (0.5)	4 (0.5)	8 (0.5)
Intra-abdominal	170 (20.0)	167 (19.9)	337 (19.9)
Lung	456 (53.6)	450 (53.6)	906 (53.6)
Pleural	5 (0.6)	8 (1.0)	13 (0.8)
Skin/Skin Structures	23 (2.7)	28 (3.3)	51 (3.0)
Urinary Tract	85 (10.0)	86 (10.2)	171 (10.1)
Vascular Catheter	9 (1.1)	6 (0.7)	15 (0.9)
Other	20 (2.4)	15 (1.8)	35 (2.1)
Gram Stain Class of Bacterial Pathogen, n (%)			
Mixed Gram	133 (15.6)	117 (13.9)	250 (14.8)
Pure Gram Negative	185 (21.8)	196 (23.3)	381 (22.5)
Pure Gram Positive	219 (25.8)	211 (25.1)	430 (25.4)
Unconfirmed Gram	28 (3.3)	45 (5.4)	73 (4.3)
Unidentified Bacterial Exposure	285 (33.5)	271 (32.3)	556 (32.9)

(continued)

Table 5.3. Illness Characteristics (concluded)
Study F1K-MC-EVAD

Variable	Drotrecogin Alfa (activated) N=850	Placebo N=840	Total N=1690
Central Laboratory Data			
Median PC Activity Level	47%	50%	48%
PC Activity Class, n (%)			
≤40%	330 (38.8)	285 (33.9)	615 (36.4)
41%–60%	240 (28.2)	227 (27.0)	467 (27.6)
61%–80%	139 (16.4)	158 (18.8)	297 (17.6)
>80%	90 (10.6)	105 (12.5)	195 (11.5)
Unknown	51 (6.0)	65 (7.7)	116 (6.9)
PC Deficient, n (%)	709 (83.4)	670 (79.8)	1379 (81.6)
Median D-Dimer Level (mg/L)	4.22	4.15	4.21
Median IL-6 Level (pg/mL)	496.96	484.00	492.00

Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; CNS = central nervous system; EENT = ear, eyes, nose, throat; IL-6 = interleukin-6; N = number of patients in treatment group; n = number of patients within treatment group; PC = Protein C; % = percentage of patients within treatment group.

Source: PC102#1#, PC103#1#, PC109#1#, PC104#1# (F1K-MC-EVAD Clinical Study Report).

5.2.3. Duration of Study Drug Exposure

Duration of study drug exposure was assessed by the duration of study drug infusion and by whether the patient received ≥95 hours of study drug infusion. Seventy-eight percent of patients received ≥95 hours of study drug infusion: 76.9% of drotrecogin alfa (activated) patients and 78.6% of placebo patients. The mean infusion duration was 85.6 hours for drotrecogin alfa (activated) patients and 86.2 hours for placebo patients.

5.3. Mortality Analyses

Table 5.4 contains a summary of the 28-day all-cause mortality rates by treatment group.

Table 5.4. 28-Day All-Cause Mortality Rates Study F1K-MC-EVAD

Treatment Group	Deceased n (%)	Survived n (%)	Total
Drotrecogin Alfa (Activated)	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%)

Abbreviations: n = number of patients within treatment group.
Source: MS208#1# (F1K-MC-EVAD Clinical Study Report).

5.3.1. Primary Analysis and Supporting Analyses

Table 5.5 contains a summary of the primary stratified 28-day all-cause mortality analysis (with pooling for underrepresented strata), and supporting analyses of stratified 28-day all-cause mortality analysis (without pooling for underrepresented strata) and nonstratified 28-day all-cause mortality analysis.

For the protocol-specified primary analysis, the primary analysis patient population was stratified into 40 strata defined by APACHE II quartile (Appendix 1), age class, and baseline Protein C activity class, characteristics that have been shown to be predictive of a poor outcome. The patient population was stratified to reduce the unwanted decrease in study power due to enrolling a patient population with varied predictive probabilities of death. On average, it is expected that 2 patients within the same stratum will have predictive probabilities of death that are more similar than 2 patients who are classified into different strata.

Forty strata were defined by preinfusion APACHE II quartile (3 to 19, 20 to 24, 25 to 29, 30 to 53), age class (<60 or ≥60 years), and baseline Protein C activity class (≤40%, 41% to 60%, 61% to 80%, >80%, or unknown). For the primary stratification, it was expected that there would be relatively few patients in a treatment group within some of the strata, particularly at the time of the planned interim analyses. Though the necessary asymptotic theory holds for the Cochran-Mantel-Haenszel test, the stability of the logit-adjusted relative risk and odds ratio confidence intervals can be questioned in the presence of sparse strata (Agresti 1990). Therefore, an algorithm was prospectively defined to pool strata prior to conducting the primary efficacy analysis.

Based on the prospectively defined primary mortality analysis, a statistically significant reduction in 28-day all-cause mortality was observed for patients receiving drotrecogin alfa (activated) compared with those receiving placebo (p=0.0054). These results represent 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 in the drotrecogin alfa (activated) treatment group compared with the placebo treatment group.

The three analyses provided in Table 5.5 yielded very similar p-values, relative risks, and odds ratios.

Table 5.5. Primary 28-Day All-Cause Mortality Analyses Study F1K-MC-EVAD

Analysis	p-Value[a]	Relative Risk	Relative Risk 95% CI	Odds Ratio	Odds Ratio 95% CI	Interaction[b]
Primary Analysis: Primary Stratification with Pooling	0.0054	0.8057	0.6950–0.9339	0.7241	0.5735–0.9143	0.2359
Primary Stratification without 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	—

Abbreviations: CI = confidence interval.

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

[b] P-value from the Breslow-Day test for homogeniety of odds ratios across strata.

Source: MS208#1#, MS308#1# (F1K-MC-EVAD Clinical Study Report).

Figure 5.2 displays the Kaplan-Meier survival curves for the primary analysis population by treatment group. The equality of the treatment group survival curves was tested using the Score test from a Cox proportional hazards model with an adjustment for preinfusion APACHE II quartile, baseline Protein C activity class, and age class (with pooling for underrepresented strata). A statistically significant increase in survival was observed for the drotrecogin alfa (activated) treatment group compared with the placebo treatment group (stratified p=0.0056, nonstratified p=0.0059).

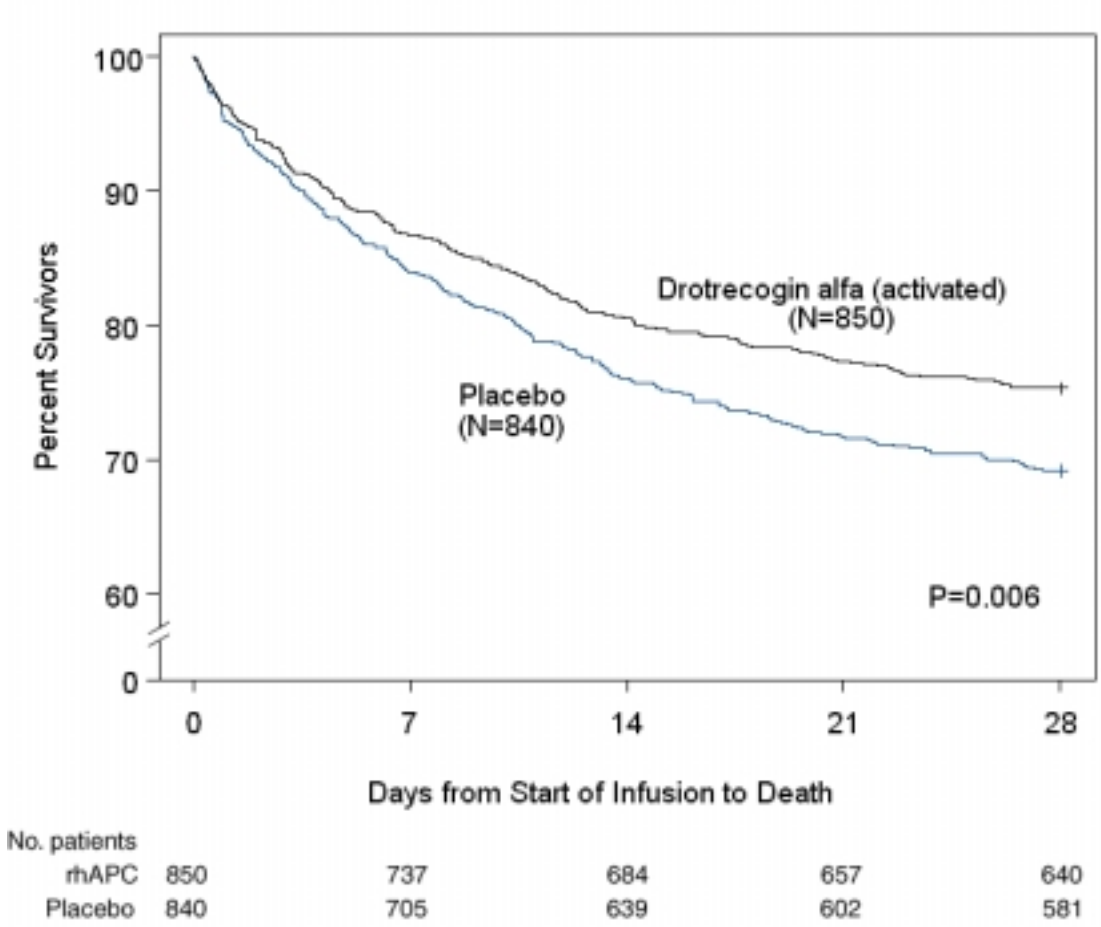


Figure 5.2. **28-day all-cause mortality analyses; Kaplan-Meier survival curves. Study F1K-MC-EVAD.**

Based on a nonstratified mortality analysis, the drotrecogin alfa (activated) treatment group experienced improved 28-day survival compared with the placebo treatment group. At 28 days, the observed mortality rates were 24.71% for the drotrecogin alfa (activated) treatment group and 30.83% for the placebo treatment group (nonstratified p=0.0049). These results represent 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 drotrecogin alfa (activated) patients compared with placebo patients.

Analyses of 28-day all-cause mortality for all enrolled patients, which included the 38 randomly assigned patients withdrawn from the study prior to receiving study drug, were also performed. In this population, a statistically significant reduction in 28-day all-cause mortality was observed in patients receiving drotrecogin alfa (activated) compared with those receiving placebo (24.80% versus 31.27%; p=0.0027). The drotrecogin alfa (activated) 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 compared with the placebo group. Analysis of 28-day all-cause mortality for all enrolled patients yielded similar p-values, relative risks, and odds ratios compared with the prospectively defined primary mortality analysis.

To examine the sensitivity of the trial conclusions to potential baseline imbalances, stratified mortality analyses were performed individually for over 75 baseline characteristics including demographics, type and site of infection, solicited historical illnesses, and clinical and biochemical measures of disease severity. In every stratified analysis, a statistically significant reduction in 28-day all-cause mortality for the drotrecogin alfa (activated) groups compared to placebo was observed. Furthermore, all analyses yielded similar p-values, relative risks, and odds ratios compared to the prospectively defined primary analysis.

5.3.2. Mortality by Protocol Version

The original Protocol F1K-MC-EVAD was approved on 7 April 1998. Under this version of the protocol, 720 patients were enrolled and received study drug (from 28 July 1998 to 15 July 1999). Amended Protocol F1K-MC-EVAD(a) was approved on 5 March 1999. Under this version of the protocol, 970 patients were enrolled and received study drug (from 6 June 1999 to 28 June 2000). The amended protocol was approved 7 months prior to the first Interim Analysis when the independent Data and Safety Monitoring Board was first unblinded to patient treatment assignments (see Table 5.1).

Under the original protocol, 109 of 360 (30.28%) placebo patients died compared to 102 of 360 (28.33%) drotrecogin alfa (activated) patients (Relative Risk: 0.9358, 95% CI 0.7457 to 1.1743). Under the amended protocol, 150 of 480 (31.25%) placebo patients died compared to 108 of 490 (22.04%) drotrecogin alfa (activated) patients (Relative Risk = 0.7053, 95% CI 0.5700 to 0.8727). The Breslow-Day interaction test p-value was 0.08. The 95% relative risk confidence intervals for the original and amendment results both include the overall relative risk estimate for the trial of 0.806, and given the moderate subgroup sample sizes, there was considerable overlap in the relative risk confidence intervals of the two subpopulations.

Investigation of potential explanations for this observed relative risk by protocol version variability beyond random chance were performed. This observed variability in relative risk estimates appears predominantly due to changes in the mix of investigators actively enrolling patients during the course of the trial. Twenty investigative sites enrolled patients only under the original protocol version. Additionally, 45 investigative sites enrolled patients only under the amended version of the protocol. When the 227 patients enrolled from these 65 investigative sites were removed from the entire population for analysis, the results for the 1463 patients from the 99 investigative sites enrolling under both protocol versions suggested no treatment-by-protocol version interaction (p=0.4987).

5.3.3. Mortality Conclusions

In summary, administration of drotrecogin alfa (activated) was associated with a statistically significant reduction in mortality for patients with severe sepsis. A statistically significant reduction in mortality was observed regardless of whether the analysis included all enrolled patients or only study drug-treated patients, or whether the analysis was stratified for baseline disease covariates.

5.4. Morbidity Results

5.4.1. Organ Dysfunction

Methods. Sequential Organ Failure Assessment (SOFA) methodology was used to assess the incidence and severity of organ dysfunction/failure in the patient population (Vincent et al. 1996, 1998). Each organ system, cardiovascular, hematology, hepatic, renal, and respiratory, was analyzed separately. The prospectively-defined primary organ failure analyses were based on data representing the degree of organ dysfunction observed from Study Day 1 through Study Day 28. Time-averaged SOFA scores for Study Days 1 through 4, 1 through 7, 1 through 14, and 1 through 28 were analyzed by treatment group.

For analysis, a patient received a score of 0, 1, 2, 3, or 4 each day based on the SOFA classification (Appendix 2). A nonsurviving patient received an organ dysfunction score of 4 (worst score) for the day of death and for every day thereafter until Study Day 28. For those days when a patient was alive but no data were available for an organ system, the last observation available was carried forward. ANOVA based on unranked data was used to draw statistical conclusions regarding treatment effects. For each organ system, the time-averaged SOFA analysis included all patients with any data.

The time to first resolution of organ dysfunction over days 1 through 7 was defined as the time patients with a SOFA score >0 at baseline took to reach 0 and was analyzed using a log-rank test. Kaplan-Meier time-to-event curves are presented. This analysis was not prospectively defined.

Prospectively-defined parameter-free day analyses included assessments of vasopressor-, ventilator-, SIRS-, ICU-, and hospital-free days. For each study day, patients were defined as having a vasopressor-, ventilator-, SIRS-, ICU-, or hospital-free day if they were alive and did not require vasopressor administration, mechanical ventilation, did not meet the SIRS criteria, were not in the ICU, or were not in the hospital, respectively (Bernard et al. 1995). For each individual parameter-free day endpoint, a patient's response was defined as the sum of the parameter-free days from Study Days 1 through 28. ANOVA based on unranked data was used to draw statistical conclusions regarding treatment effect.

SOFA Results – Primary Analysis Population. Figure 5.3 contains a summary of SOFA score analyses by treatment group and organ system.

Cardiovascular. The drotrecogin alfa (activated) treatment group had a statistically significantly lower (better) mean 28-day time-averaged cardiovascular SOFA score relative to the placebo treatment group (p=0.009). In addition, the drotrecogin alfa (activated) treatment group had a statistically significantly lower mean time-averaged cardiovascular SOFA scores for Study Days 1 through 4, 1 through 7, and 1 through 14 (p=0.023, p=0.025, and p=0.033, respectively) relative to the placebo treatment group.

Respiratory. The drotrecogin alfa (activated) treatment group had a statistically significantly lower (better) mean 28-day time-averaged respiratory SOFA score (p=0.023) and a lower mean time-averaged SOFA score for Study Days 1 through 14 that approached statistical significance relative to the placebo treatment group (p=0.073).

Renal. The drotrecogin alfa (activated) treatment group had a lower (better) mean 28-day time-averaged renal SOFA score that approached statistical significance relative to the placebo treatment group (p=0.077).

Hematologic. The drotrecogin alfa (activated) treatment group had a lower (better) mean 28-day time-averaged hematologic SOFA score that approached statistical significance relative to the placebo treatment group (p=0.057).

Hepatic. There were no statistically significant differences between the two treatment groups in time-averaged SOFA scores for any time frame.

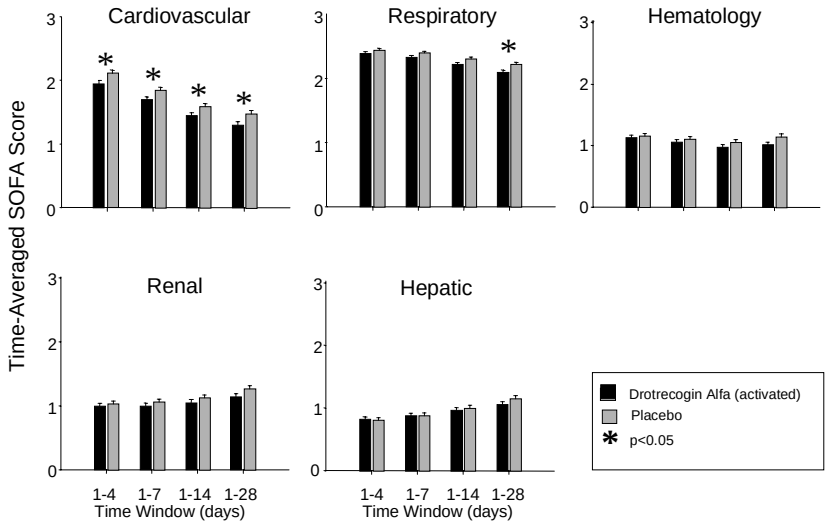


Figure 5.3. Time-averaged SOFA scores for patients in the primary analysis population. Study F1K-MC-EVAD.

Time to First Resolution of Organ Dysfunction Analyses. Two of the SOFA organ systems evaluated, cardiovascular and respiratory, resolved significantly more rapidly in drotrecogin alfa (activated) patients with organ dysfunction at baseline compared with

placebo patients with organ dysfunction at baseline (both p=0.009). Time to resolution of organ dysfunction was defined as the time patients with a SOFA score >0 took to reach a SOFA score of 0. There was no difference between the two treatment groups in the time to resolution of renal, hepatic, and hematologic organ dysfunction. Figure 5.4 and Figure 5.5 show the time to first resolution of cardiovascular organ dysfunction and the time to first resolution of respiratory organ dysfunction, respectively.

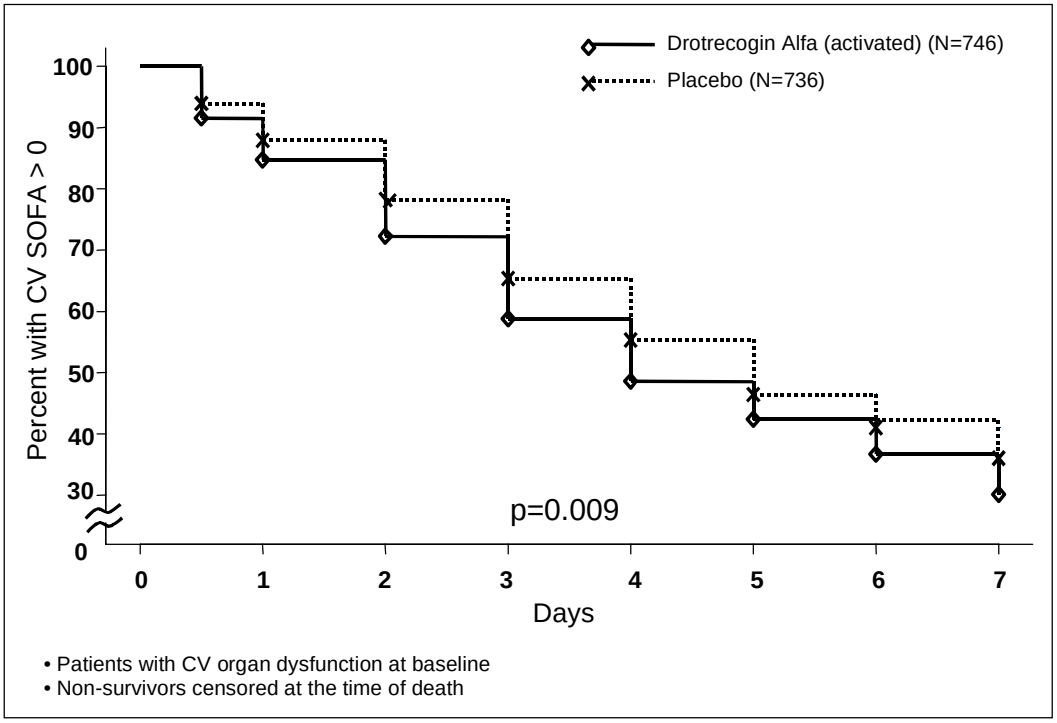


Figure 5.4. **Time to first resolution of cardiovascular organ dysfunction for patients in the primary analysis population. Study F1K-MC-EVAD.**

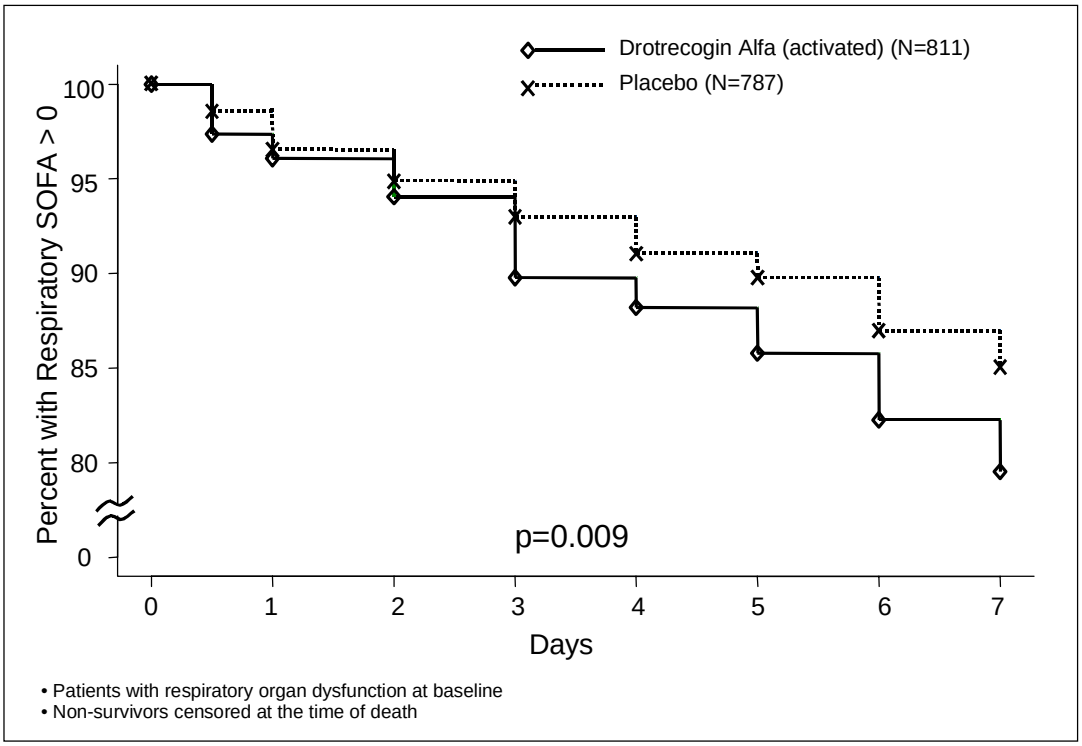


Figure 5.5. **Time to first resolution of respiratory organ dysfunction for patients in the primary analysis population. Study F1K-MC-EVAD.**

Resource Use Results. In line with the observed effects on cardiovascular organ function, the drotrecogin alfa (activated) treatment group had a statistically significant increase (improvement) in vasopressor-free days compared with the placebo treatment group (mean 20.06 days versus 18.78 days; p=0.014).

In addition, in line with the observed effects on respiratory organ function, the drotrecogin alfa (activated) treatment group had a statistically significant increase (improvement) in ventilator-free days compared with the placebo treatment group (mean 14.33 days versus 13.24 days; p=0.049).

There were no statistically significant differences between the two treatment groups in SIRS-, ICU-, or hospital-free days. However, for almost all study days a higher percentage of patients in the drotrecogin alfa (activated) group were alive, alive and not in the intensive care unit, or alive and not in the hospital. Figure 5.6 shows patient location for all patients over the 28-day study period by treatment group (where percent of patients corresponds to the area under the curve). In this figure, letters "a" through "f" correspond to the following:

a. Percent of drotrecogin alfa (activated) patients alive on each study day.

b. Percent of placebo patients alive on each study day.

c. Percent of drotrecogin alfa (activated) patients alive and out of the ICU on each study day.

d. Percent of placebo patients alive and out of the ICU on each study day.

e. Percent of drotrecogin alfa (activated) patients alive and out of the hospital on each study day.

f. Percent of placebo patients alive and out of the hospital on each study day.

Lines "a" and "b" represent the Kaplan-Meier survival curves for the drotrecogin alfa activated and placebo groups, respectively. As previously shown, the survival benefit associated with drotrecogin alfa (activated) was evident by four days following the start of study drug administration. Lines "c" and "d" represent the percent of patients alive and not in the intensive care unit (ICU) for the drotrecogin alfa activated and placebo groups, respectively. For almost all study days, a higher percentage of patients in the drotrecogin alfa (activated) group were alive and not in the intensive care unit. Lines "e" and "f" represent the percent of patients alive and not in the hospital for the drotrecogin alfa activated and placebo groups, respectively. For almost all study days, a higher percentage of patients in the drotrecogin alfa (activated) group were alive and not in the hospital.

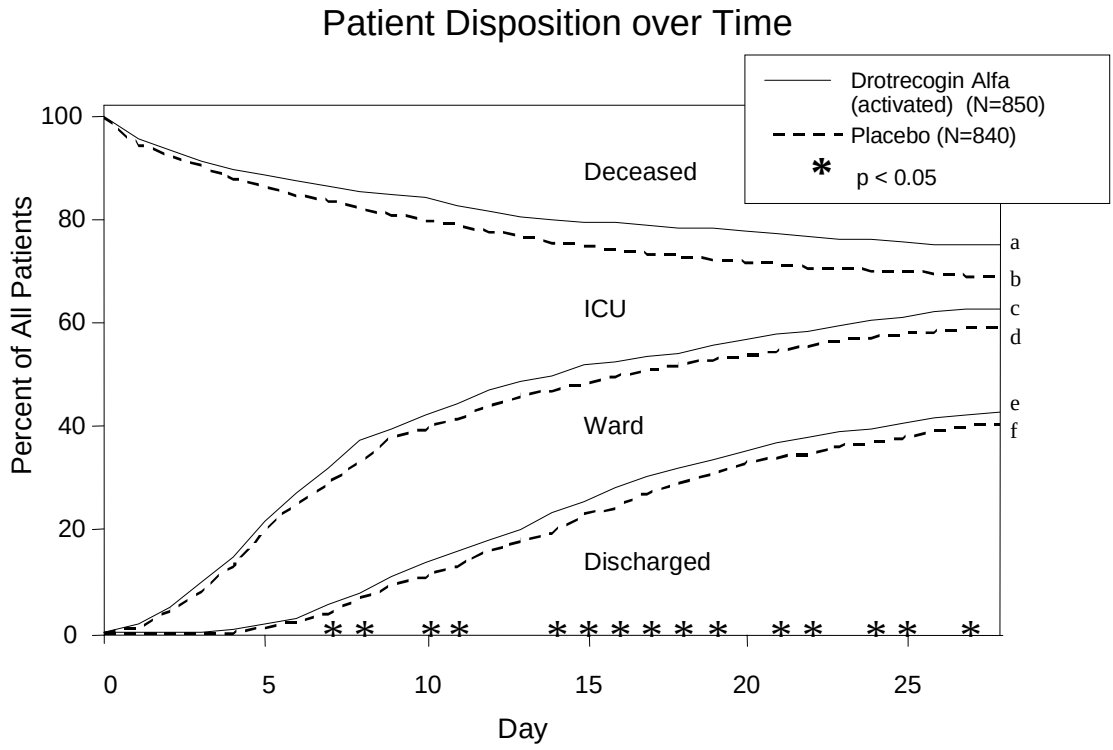


Figure 5.6. **Patient disposition over time for patients in the primary analysis population. Study F1K-MC-EVAD.**

P-values comparing treatment groups are based on a daily ANOVA analysis with patient location treated as an ordered variable (Deceased=0, ICU=1, Ward=2, Discharged=3).

From Study Day 7 forward, a higher percentage of drotrecogin alfa (activated) treated patients were alive and discharged from the hospital and alive and out of the ICU compared to placebo-treated patients.

5.4.2 Patient Location and Functional Status for Survivors

At Study Day 28, an assessment was made regarding patient location (home-receiving no support, home-receiving paid professional support, home-receiving unpaid support, other acute care hospital, skilled nursing facility). The association between treatment and patient location was assessed using a Pearson's chi-square test. For this analysis, treatment groups are not defined only by randomization, but are also conditioned on patient survival.

Figure 5.7 presents a summary by treatment group of patient location on Study Day 28. There was no statistically significant association between the two treatment groups in

patient location on Study Day 28. Compared with placebo survivors, a similar proportion of drotrecogin alfa (activated) survivors were at home on Study Day 28.

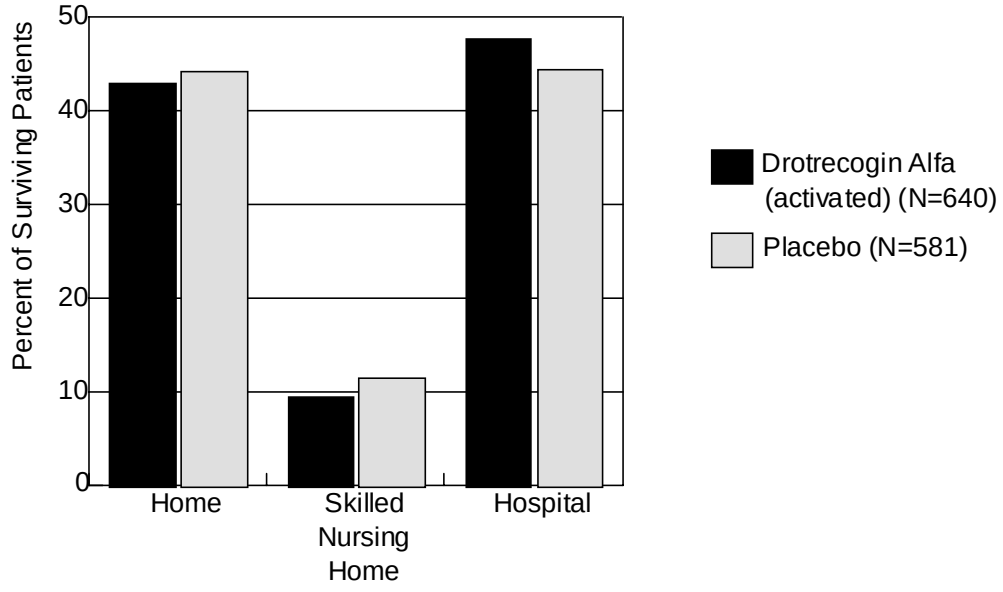


Figure 5.7. Patient location at Study Day 28 for surviving patients. Study F1K-MC-EVAD.

Activities of Daily Living Results. At Study Day 28, functional status was assessed using the Index of Independence in Activities of Daily Living (ADL) scale. Patients were measured on their highest level of ability to perform the following activities: bathing, dressing, toileting, transferring out of bed, bowel and bladder control, and feeding. For each activity, patients received a score of 0 or 1. A score of 0 indicated that the patient was independent and required little or no assistance to perform the activity; a score of 1 indicated that the patient was dependent and required significant assistance or was unable to perform the activity. For each activity, treatment groups were assessed using Pearson's chi-square tests. For this analysis, treatment groups are not defined only by randomization, but are also conditioned on patient survival.

Figure 5.8 shows the percentage of 28-day survivors in each treatment group who were considered independent for each component of the ADL score at Study Day 28. There was no statistically significant difference between treatment groups in the percentage of survivors who were assessed as independent based on Pearson's chi-square tests.

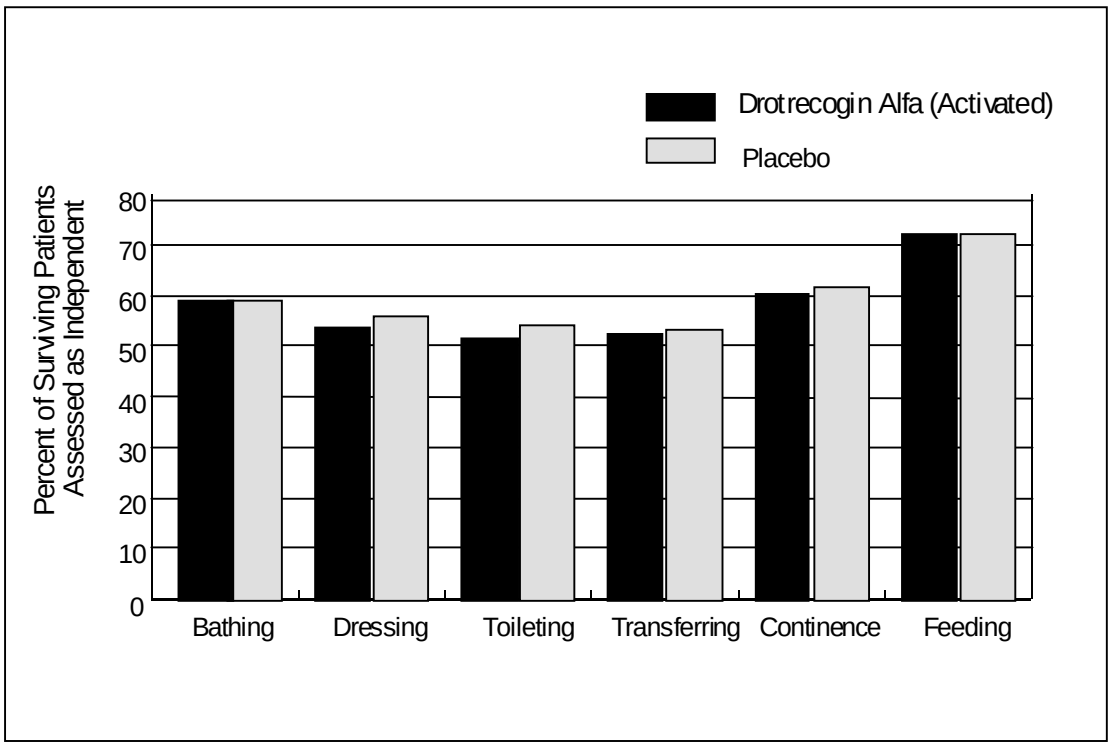


Figure 5.8. **The percentage of surviving patients considered independent for each component of the ADL scale at Study Day 28. Study F1K-MC-EVAD.**

5. 5. Overall Efficacy Conclusions

The key efficacy findings of the pivotal Study F1K-MC-EVAD are as follows:

- Drotrecogin alfa (activated) administration at a dose of 24 μg/kg/hr for 96 hours reduces 28-day all-cause mortality in patients with sepsis and associated acute organ dysfunction (severe sepsis) compared with placebo.
- Drotrecogin alfa (activated) patients experienced more days alive without the need for vasopressor support and mechanical ventilation compared with placebo patients.
- On almost all study days, a higher percentage of drotrecogin alfa (activated) treated patients were alive and discharged from the hospital and alive and out of the ICU compared to placebo-treated patients.
- Drotrecogin alfa (activated) survivors had similar functional status at Study Day 28 compared with placebo survivors as demonstrated by patient location and the ADL assessment.

6. Pharmacokinetics in Adult Patients with Severe Sepsis

6.1. Overview

The pharmacokinetic profile of drotrecogin alfa (activated) in the Phase 2/3 studies was characterized primarily by plasma concentration at steady-state (C_{ss}) and plasma clearance (Cl_p). Volume of distribution was not estimated in sepsis patients, and elimination rates were evaluated as the postinfusion time required for plasma concentrations to fall below the detection limit (t_{last}).

Results of the Phase 2/3 studies indicate that Cl_p in patients with severe sepsis is higher than that in normal healthy subjects, which produces lower C_{ss} in sepsis patients than in healthy subjects. Plasma clearance (L/hr) increased with increasing body weight. Weight-adjusted plasma clearance (L/kg/hr) was statistically significantly affected by age, gender, hepatic function, and renal function, but the effects of these factors were small and were judged to be not clinically significant. Plasma clearance did not depend on ethnicity, estrogen status, or heparin status. Therefore dose adjustment, other than by body weight, is not recommended in any patient subpopulation.

Study F1K-MC-EVAA. Plasma Activated Protein C concentrations were measured using an amidolytic immunocapture assay validated over an Activated Protein C concentration range of 5 to 100 ng/mL. Of the 131 patients enrolled, 90 received drotrecogin alfa (activated) and 41 received placebo.

Study F1K-MC-EVAD. Plasma Activated Protein C concentrations in 342 drotrecogin alfa (activated)-treated patients were measured using an amidolytic immunocapture assay validated over an Activated Protein C concentration range of 10 to 100 ng/mL. These patients comprised a subset of North American patients enrolled in the study. Of these 342 patients, 326 provided evaluable steady-state concentrations on which to base pharmacokinetic assessments.

6.2. Parameter Estimates

Table 6.1 contains a listing of C_{ss} and Cl_p derived during Studies F1K-MC-EVAA and F1K-MC-EVAD.

Table 6.1. Steady-State Plasma Activated Protein C Concentrations and Drotrecogin Alfa (Activated) Plasma Clearance Measured in Sepsis Patients During Phase 2 and 3 Clinical Trials

Infusion Rate and Duration	C_{ss} (ng/mL) Actual	C_{ss} (ng/mL) Normalized to 24 μg/kg/hr[a]	Cl_p (L/hr)
Phase 2: F1K-MC-EVAA[b]			
12 μg/kg/hr (N=74 samples/23 patients)	25.9 ± 8.6	51.8 ± 17.2	44.6 ± 28.0
18 μg/kg/hr (N=71 samples/25 patients)	41.4 ± 17.8	55.2 ± 23.7	50.1 ± 53.3
24 μg/kg/hr (N=58 samples/21 patients)	60.8 ± 26.3	60.8 ± 26.3	44.6 ± 31.4
30 μg/kg/hr (N=17 samples/10 patients)	79.4 ± 36.8	63.5 ± 29.4	36.2 ± 12.0
All infusions (N=220 samples/79 patients)	NC	NC	45.8 ± 38.1
Phase 3: F1K-MC-EVAD			
24 μg/kg/hr for 96 hr (N=843 samples/326 patients)	51.5[c]	51.5[c]	41.8[c]

Results are expressed as mean ± SD.

Abbreviations: Cl_p = plasma clearance; C_{ss} = steady-state plasma Activated Protein C concentration; NC = not calculated; SD = standard deviation.

[a] Normalized C_{ss} = Actual C_{ss} • ($^{24\ \mu g/kg/hr}/_{Infusion\ rate}$).

[b] Results from Study F1K-MC-EVAA for 12, 18, and 24 μg/kg/hr are combined 48 hr + 96 hr; 30 μg/kg/hr was infused for only 48 hours.

[c] SD not provided in original study reports.

Sources: Table EVAA.11.28, Table EVAA.11.29, Table EVAA.11.30, Table EVAD.14.138, and Table EVAD.14.139 (F1K-MC-EVAA Clinical Study Report).

Steady-State Plasma Concentrations of Activated Protein C in Patients with Severe Sepsis. Plasma concentrations of endogenous Activated Protein C in sepsis patients were usually below the quantitation limit, and therefore did not significantly affect the characterization of drotrecogin alfa (activated) pharmacokinetics during treatment. In the Phase 2 study F1K-MC-EVAA, C_{ss} was independent of infusion duration but was proportional to infusion rate. In the Phase 3 study F1K-MC-EVAD, C_{ss} in 75% of patients ranged from 10.1 ng/mL to 59.2 ng/mL, with a median of 43.2 ng/mL and a mean of 51.5 ng/mL (N=843 samples from 326 patients) (Table 6.2).

Table 6.2. Steady-State Plasma Activated Protein C Concentrations and Plasma Clearance During Intravenous Drotrecogin Alfa (Activated) Infusion at a Rate of 24 μg/kg/hr for 96 Hours During Phase 3 Study F1K-MC-EVAD

	C_{ss} (ng/mL)	Cl_p (L/hr)
1st Quartile	32.4	26.8
Median	43.2	40.1
Mean	51.5	41.8
3rd Quartile	59.2	52.0
Minimum	10.1	6.6
Maximum	402.5	157.7
Number of samples	843	326
Number of patients	326	326

Abbreviations: Cl_p = plasma clearance; C_{ss} = steady-state plasma Activated Protein C concentration.
Source: Table EVAD.14.138 for C_{ss}; Table EVAD.14.139 for Cl_p (F1K-MC-EVAD Clinical Study Report).

Clearance of Drotrecogin Alfa (Activated) in Patients with Severe Sepsis. The estimated Cl_p of 45.8 ± 38.1 L/hr (mean ± SD, N=220 samples from 79 patients) in Study F1K-MC-EVAA was 63% higher than the estimated Cl_p of 28.1 ± 8.6 L/hr in 190 doses to healthy subjects during the Phase 1 studies (Table 3.1). The median and mean Cl_p measured in the Phase 3 study F1K-MC-EVAD were 40.1 L/hr and 41.8 L/hr, respectively (N=326 patients), and were therefore comparable to each other and to the mean Cl_p of 45.8 L/hr from Study F1K-MC-EVAA. Statistically significant differences in Cl_p associated with age, gender, renal function, and liver function tests were <30%, fell within the interquartile range of Cl_p, and did not prompt a recommendation for dose adjustment based on these parameters. The effects of these covariates are discussed in Section 6.3, "Pharmacokinetics in Special Populations."

Plasma clearance in Study F1K-MC-EVAD was not statistically significantly affected by estrogen status, ethnicity, baseline Protein C activity level, or baseline IL-6 level.

Elimination of Drotrecogin Alfa (activated) in Patients with Severe Sepsis. Elimination of drotrecogin alfa (activated) was rapid in sepsis patients. In the Phase 2 study F1K-MC-EVAA, the mean time for plasma Activated Protein C concentrations to fall below 5 ng/mL was generally less than 4 hours. In the Phase 3 study F1K-MC-EVAD, plasma concentrations in most patients were below 10 ng/mL by 2 hours after stopping infusion. These results in patients with severe sepsis are consistent with each other and with the short half-lives of 13 minutes and 1.6 hours in healthy subjects and

indicate that drotrecogin alfa (activated) is eliminated as rapidly in patients with severe sepsis as it is in healthy subjects.

6.3. Pharmacokinetics in Special Populations

The primary purpose of assessing drotrecogin alfa (activated) pharmacokinetics in special populations was to identify demographic variables and disease conditions that may require dosage adjustment in patients with severe sepsis. Covariates that influenced drotrecogin alfa (activated) pharmacokinetics during Phase 1 studies were considered in the design of the Phase 2 study F1K-MC-EVAA. The totality of pharmacokinetic insight gained from Phase 1 and Phase 2 studies was used to evaluate dosage adjustment in the Phase 3 study F1K-MC-EVAD. Based on all studies conducted to date, body weight is the only clinical or demographic covariate that requires a dosage adjustment.

Effect of Body Weight. Plasma clearance increased with increasing body weight. Body weight was found to be a statistically significant covariate ($p<0.001$) of Cl_p in patients with severe sepsis in the Phase 2 study F1K-MC-EVAA. When included as one of six covariates in study F1K-MC-EVAA, body weight accounted for approximately 23.8% of the observed interpatient variability. Weight-based adjustment of drotrecogin alfa (activated) infusion rates in patients with severe sepsis substantially reduces interpatient variability in C_{ss}, which is the basis for the recommended weight-based infusion rate of 24 μg/kg/hr.

Effect of Age. In the Phase 2 study F1K-MC-EVAA, C_{ss} in patients >65 years old was approximately 23% higher ($p=0.006$) than those in patients ≤65 years old, but there was considerable overlap between these subgroups. When included as one of six covariates, age accounted for approximately 12.9% ($p=0.001$) of the observed interpatient variability in Cl_p. Based on the relatively small contribution of age in explaining the overall pharmacokinetic variability of drotrecogin alfa (activated) in patients with severe sepsis, and the lack of any age-associated safety issues in the Phase 2 study, age-based dosage adjustment was not recommended for the Phase 3 study F1K-MC-EVAD.

In the Phase 3 study F1K-MC-EVAD, mean Cl_p in patients ≥60 years old was 19.7% lower (nonweight-adjusted; $p<0.001$) and 14.8% lower (weight-adjusted; $p<0.001$) than that in patients <60 years old. Likewise, mean Cl_p in patients ≥65 years was 20.5% lower (nonweight-adjusted; $p<0.001$) and 13.9% lower (weight-adjusted; $p<0.001$) than that in patients <65 years old.

Based on the small magnitude of difference between Cl_p in elderly and nonelderly patients, the relatively small contribution of age in explaining the overall pharmacokinetic variability of drotrecogin alfa (activated) in patients with severe sepsis, and the lack of any age-associated safety concerns in patients with severe sepsis, age-based dosage adjustment is not recommended.

Effect of Gender and Estrogen Status. In the Phase 2 study F1K-MC-EVAA, gender (female versus male, p=0.195) and estrogen status (p=0.586) had no discernable effect on the pharmacokinetics of drotrecogin alfa (activated) in patients with severe sepsis.

In the Phase 3 study F1K-MC-EVAD, Cl_p in female patients was 22.9% lower (nonweight-adjusted; p<0.001) and 14.8% lower (weight-adjusted; p<0.001) than that in male patients. Plasma clearance did not depend on estrogen status.

Based on the small magnitude of difference between Cl_p in male and female patient and on the lack of any gender-associated safety concerns in patients with severe sepsis, gender is not a clinically significant covariate and therefore does not require dose adjustment.

Effect of Renal Impairment. In the Phase 3 study F1K-MC-EVAD, mean Cl_p in patients with a Cockroft-Gault creatinine clearance of <20 mL/min was 34.9% lower (nonweight-adjusted; p≤0.001) and 23.7% lower (weight-adjusted; p≤0.001) than that in patients with a Cockroft-Gault creatinine clearance of >50 mL/min.

Mean weight-adjusted Cl_p was 27.4% lower in patients with a baseline renal SOFA score of 3 compared with those with a baseline renal SOFA score of 0 (p=0.001). There was no statistically significant relationship between baseline renal SOFA score and nonweight-adjusted Cl_p.

Based on the magnitude of difference in Cl_p between normal and renally-impaired patients on dialysis, and on the lack of any safety concerns associated with renal impairment, dosage adjustment based on renal function is not recommended.

Effect of Abnormal Liver Function. In the Phase 3 study F1K-MC-EVAD, mean Cl_p in patients with baseline aspartate aminotransferase (AST) levels more than three times the upper limit of normal was lower by 25.2% (nonweight-adjusted; p<0.001) and 23.7% (weight-adjusted; p<0.001) than that in patients with normal baseline AST levels. Mean Cl_p in patients with baseline alanine aminotransferase (ALT) levels more than three times the upper limit of normal was 30.9% lower (nonweight-adjusted; p<0.001) and 26.7% lower (weight-adjusted; p<0.001) than that in patients with normal baseline ALT levels. Plasma clearance did not significantly depend on baseline hepatic SOFA score.

Based on the magnitude of difference in Cl_p between patients with normal liver function and those with abnormal liver function tests, and on the lack of any safety concerns associated with the use of drotrecogin alfa (activated) in patients with abnormal liver function tests, dosage adjustment based on liver function is not recommended.

Effect of Ethnicity. In the Phase 3 study F1K-MC-EVAD, there was no effect of ethnicity on Cl_p. Based on this result, dosage adjustment based on ethnic origin is not recommended.

Effect of Alcohol and Smoking. In the Phase 2 study F1K-MC-EVAA, neither alcohol ingestion (p=0.99) nor smoking (p=0.389) significantly affected C_{ss} in patients with

severe sepsis. Based on these results, dosage adjustment based on drinking or smoking habits is not recommended.

Effect of Heparin A patient was classified as being exposed to heparin if the patient received any dose of unfractionated or low molecular weight heparin by any route (excluding the use of heparin to maintain vascular catheter patency) during the study drug infusion or the next calendar day after the end of the infusion. Dose and route of administration of heparin were not collected.

In Study F1K-MC-EVAD, Cl_p was not significantly affected by heparin coadministration.

Effect of APACHE II Score In Study F1K-MC-EVAD, the lowest mean nonweight-adjusted Cl_p occurred in patients in the fourth APACHE II quartile of 30 to 50, and was 19.7% lower than that in patients in the first APACHE II quartile of 3 to 19 (p=0.041). There was no statistically significant relationship between preinfusion APACHE II quartile and weight-adjusted Cl_p. Based on these results, dosage adjustment based on disease severity is not recommended.

Effect of Coagulation Biomarkers In Study F1K-MC-EVAD, there was a statistically significant relationship between baseline hematology SOFA score (based on platelet count) and weight-adjusted Cl_p (p=0.037). Weight-adjusted Cl_p was lowest in 1 patient with a baseline hematology SOFA score of 4 and highest in the patients with a baseline hematology SOFA score of 3. Nonweight-adjusted Cl_p did not depend on the baseline hematology SOFA score.

Compared to patients with normal baseline PT, mean Cl_p was 13.9% lower (nonweight-adjusted; p=0.035) and 14.8% lower (weight-adjusted; p=0.008) in patients with baseline PT that was more than 1.2 times the upper limit of normal (Study F1K-MC-EVAD).

Plasma clearance was not statistically significantly associated with baseline whole blood APTT, Protein C activity, or IL-6 (Study F1K-MC-EVAD).

Based on these results, dosage adjustment based on coagulation biomarkers is not recommended.

Pharmacokinetics in Patients with Multiple Covariates. Dosage adjustment is not recommended in patients with multiple covariates that affect plasma clearance.

In Study F1K-MC-EVAD, the four factors that statistically significantly decreased Cl_p were: Being female, being ≥65 years old, having renal impairment, and having hepatic impairment. None of these individually decreased Cl_p enough to justify dosage adjustment, but the presence of several factors at once could conceivably produce a multiplicative decrease in Cl_p and a commensurate increase in C_{ss}.

The impact of multiple factors was assessed by comparing C_{ss} in subgroups of F1K-MC-EVAD patients who had increasing numbers of the four factors that affect Cl_p

(Table 6.3). As the number of factors increased, mean C_{ss} increased but did so to a maximum of only 77.9 ng/mL, which is less than 50% higher than the mean C_{ss} in all patients. In the most extreme subgroup of two elderly female patients with renal and hepatic impairment, the mean C_{ss} of 75.3 ng/mL was only 40% higher than the mean C_{ss} in all patients, and the individual C_{ss} of 51.0 ng/mL in one of these patients was nearly identical to the mean in all patients. Furthermore, the range of individual C_{ss} in patients with more factors is consistently fully encompassed by the range of C_{ss} in patients with fewer factors, which makes the number of factors that affect Cl_p an unreliable predictor of C_{ss} in individual patients.

For these reasons, dosage adjustment is not recommended based on any combination of factors known to affect Cl_p.

Table 6.3. Steady-State Plasma Concentrations (C_{ss}) in Study F1K-MC-EVAD Patients with Multiple Factors that Affect Plasma Clearance

		C_{ss} (ng/mL)	
(Number of) Covariate(s)	N	Mean	Range
(0) None (all patients)	326	53.7 ± 34.1a	14.1 - 391
(1) Females	141	60.4	15.8 - 391
(2) Females, ≥65 yr	64	62.9	15.8 - 193
(3) Females, ≥65 yr, Cl_{cr} <20 mL/min	10	67.6	15.8 - 138
(3) Females, ≥65 yr, ALT or AST >3× normal	13	77.9	32.9 - 131
(4) Females, ≥65 yr: Cl_{cr} <20 mL/min and ALT or AST >3× normal	2	75.3[a]	51.0, 99.6[b]

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransaminase; Cl_{cr} = creatinine clearance; C_{ss} = clearance at steady-state.

[a] Mean ± Standard Deviation.

[b] Individual values.

6.4. Conclusions

- Plasma clearance (Cl_p) was lower and steady-state plasma concentrations (C_{ss}) were higher in healthy subjects than in patients with severe sepsis.
- The pharmacokinetics of drotrecogin alfa (activated) were linear in patients with severe sepsis.

- Steady-state plasma concentrations were reached rapidly after the start of infusion, and plasma concentrations declined rapidly after the infusion was stopped.
- Plasma clearance increased with increasing body weight, and therefore weight-based dosage adjustment is appropriate.
- No dose adjustment is recommended based on age, gender, renal function, or hepatic function, nor is dose adjustment indicated based on Protein C levels.

7. Pharmacodynamics in Patients with Severe Sepsis

In Study F1K-MC-EVAD, 14 biomarkers were measured and analyzed to characterize the extent of abnormalities in coagulation and fibrinolysis, and the degree of inflammation in the patient with severe sepsis. All determinations were performed through central laboratory facilities to ensure uniformity of results. The 14 biomarkers measured were the following:

Markers of Coagulation

Platelet count: A measure of the number of circulating platelets. A decreased platelet count may be due to increased destruction, as may occur with disseminated intravascular coagulation (DIC), or decreased production.

Prothrombin time (PT): A global test of the coagulation cascade that is sensitive to the extrinsic (tissue factor) pathway of coagulation. A prolonged PT usually results from a decrease in the concentration of one or more of the coagulation factors involved in the extrinsic pathway of coagulation. A prolonged PT is commonly seen with oral anticoagulant therapy, liver disease, vitamin K deficiency, and consumptive coagulopathies, including DIC.

Activated partial thromboplastin time (APTT): A global test of the coagulation cascade that is sensitive to the intrinsic pathway of coagulation. A prolonged APTT usually results from a decrease in the concentration of one or more of the coagulation factors involved in the intrinsic pathway of coagulation. A prolonged APTT is commonly seen with heparin therapy, hemophilia, and consumptive coagulopathies, including DIC.

Protein C: The zymogen of endogenous Activated Protein C. Once converted to the active form, it is responsible for the inactivation of two major cofactors (Factors Va and VIIIa) involved in the coagulation cascade. Reduced levels of Protein C are commonly seen in liver disease, oral anticoagulant therapy, vitamin K deficiency, and consumptive coagulopathies, such as DIC. The decreased levels in consumptive coagulopathies may result from utilization during the regulation of enhanced coagulation activation or proteolysis by active enzymes such as neutrophil elastase.

Protein S: A vitamin-K-dependent protein that functions as a cofactor (catalyst) for Activated Protein C. Protein S enhances the inactivation of Factors Va and VIIIa by Activated Protein C. A reduced level of Protein S may be seen in liver disease, oral anticoagulant therapy, vitamin K deficiency, and consumptive coagulopathies, such as DIC. The decreased levels in consumptive coagulopathies may result from utilization during the regulation of enhanced coagulation activation or proteolysis by active enzymes such as neutrophil elastase and thrombin.

Antithrombin: A serine protease inhibitor that blocks the enzymatic activity of Factor Xa and thrombin, two key enzymes of the coagulation cascade. Reduced levels

of antithrombin are commonly seen in liver disease and consumptive coagulopathies, such as DIC. The decreased levels in consumptive coagulopathies may result from utilization during the regulation of enhanced coagulation activation or proteolysis by active enzymes such as neutrophil elastase.

Markers of Thrombin Generation

D-dimer (quantitative): A specific type of fibrin degradation product produced by the plasmin-mediated breakdown (lysis) of a stabilized fibrin clot. Increased levels of D-dimer reflect both increased fibrin formation (increased thrombin activity) and in vivo fibrinolysis (increased plasmin activity).

Prothrombin fragment F1.2 (F1.2): A peptide released from prothrombin when it is converted to thrombin. The half-life of F1.2 in circulation is relatively short. Thus, the concentration of F1.2 in a plasma sample is a measure of the magnitude of ongoing thrombin formation; an increased concentration of F1.2 indicates increased thrombin formation.

Thrombin-antithrombin complex (TATc): An enzyme-inhibitor complex formed when thrombin is inactivated by antithrombin. TATc has a short half-life in the circulation. Thus, an increased concentration of TATc indicates increased thrombin formation with subsequent inhibition.

Markers of Fibrinolysis

D-dimer (quantitative): see under Markers of Thrombin Generation for description.

Plasminogen: The zymogen of plasmin. When converted to its active form by tissue plasminogen activator or urokinase, plasmin is capable of digesting fibrin clots. Plasmin can also degrade a variety of matrix and cell surface proteins. Decreased levels of plasminogen may be seen in liver disease and consumptive coagulopathies, such as DIC.

α_2-Antiplasmin: A serine protease inhibitor that blocks the enzymatic activity of plasmin. A decreased level of α_2-antiplasmin may be seen in liver disease or in association with increased activation of plasmin.

Plasminogen activator inhibitor-1 (PAI-1): A serine protease inhibitor that blocks the enzymatic activity of tissue plasminogen activator and Activated Protein C. PAI-1 is synthesized and released by endothelial cells and is an acute phase reactant. Increased levels of PAI-1 are associated with impaired fibrinolysis due to the inhibition of tissue plasminogen activator. PAI-1 also inhibits Activated Protein C, forming a 1:1 enzyme-inhibitor complex.

Thrombin activatable fibrinolysis inhibitor (TAFI): A carboxypeptidase that cleaves carboxy-terminal lysine amino acids from peptide chains. These lysines often form part of the recognition site for plasmin. Thus, TAFI inhibits fibrinolysis by

preventing the interaction between plasmin and its target proteins. TAFI is activated by thrombin. Decreased levels of TAFI may be seen in consumptive coagulopathies.

Marker of Inflammation

Interleukin-6 (IL-6): A proinflammatory cytokine released during the systemic inflammatory response. The concentration of IL-6 correlates with prognosis; the higher the level of IL-6 at presentation, the higher the risk of mortality.

Biomarker levels were determined on the following study days:

- D-dimer, IL-6, PT, APTT, Protein C, Protein S, and antithrombin levels were determined at baseline, daily for Study Days 1 through 7, and at Study Days 14 and 28. Only baseline and Study Day 1 through 7 data are presented here.
- F1.2, TATc, PAI-1, TAFI, α_2-antiplasmin, and plasminogen levels were determined at baseline and at Study Days 1, 2, 4, and 5 for a subset of patients.
- Platelet count was determined at baseline and at Study Days 4, 6, 14, and 28.

7.1. Baseline Biomarker Data

At study entry, almost all patients had generalized procoagulant and inflammatory responses to infection, as evidenced by elevated IL-6, D-dimer, PT in 98.5%, 99.7%, and 93.4% of the patients respectively (Table 7.1).

Prolongation of PT was more prevalent than prolongation of APTT at baseline. This is consistent with the understanding that the coagulation activation during the host response to infection is mainly via the tissue pathway (extrinsic pathway). Baseline platelet counts were below the lower limit of normal in only one-third of the patients, consistent with the data in the Phase 2 study F1K-MC-EVAA and the published literature. Thrombocytopenia in a patient with severe sepsis is often associated with the development of overt disseminated intravascular coagulation.

Markers of thrombin generation (F1.2 and TATc) were elevated in almost all patients In contrast, markers of abnormal fibrinolysis (elevation of PAI-1, consumption of TAFI, plasminogen and α_2-antiplasmin) were present in about half of the patients. Acquired deficiency (below the lower limit of normal) of the anticoagulant factors, Protein C, Protein S and antithrombin was highly prevalent at baseline, occurring in 87.6%, 77.8% and 81.7% of the patients respectively. These findings are consistent with the presence of a significant coagulopathy in most patients at baseline.

Table 7.1. Summary of Preinfusion Biomarker Data
Primary Analysis Population
Study F1K-MC-EVAD

Biomarker	LLN	ULN	No. of Patients with Data	Median Value	Below LLN n	Below LLN (%)	Within Range n	Within Range (%)	Above ULN n	Above ULN (%)
Platelet Count (GI/L)	140	400	1419	182	434	(30.6)	888	(62.6)	97	(6.8)
PT (s)	10.6	14.5	1558	18.7	0		103	(6.6)	1455	(93.4)
APTT (s)	21.0	39.0	1561	42.6	0		576	(36.9)	985	(63.1)
D-Dimer (mg/L)	0.00	0.39	1550	4.21	0		4	(0.3)	1546	(99.7)
Protein C (% activity)	81	173	1574	48	1379	(87.6)	193	(12.3)	2	(0.1)
Antithrombin (% activity)	80	120	1558	59	1273	(81.7)	270	(17.3)	15	(1.0)
Protein S (% activity)	60	155	1541	36	1199	(77.8)	341	(22.1)	1	(0.1)
IL-6 (pg/mL)	0.38	10.09	1635	492.00	0		24	(1.5)	1611	(98.5)
F1.2 (nmol/L)	0.44	1.10	396	1.76	8	(2.0)	81	(20.5)	307	(77.5)
TATc (µg/L)	1.00	4.10	397	11.00	0		18	(4.5)	379	(95.5)
PAI-1 (AU/mL)	4.00	37.80	298	34.00	2	(0.7)	165	(55.4)	131	(44.0)
TAFI (µg/mL)	2.80	9.20	319	4.60	56	(17.6)	243	(76.2)	20	(6.3)
α_2-Antiplasmin (% activity)	100	160	319	98	163	(51.1)	156	(48.9)	0	
Plasminogen (% activity)	64	111	316	61	174	(55.1)	134	(42.4)	8	(2.5)

Abbreviations: APTT = activated partial thromboplastin time; F1.2 = prothrombin fragment F1.2; IL-6 = interleukin-6; LLN = lower limit of normal; n = number of patients with a value in the range; No. =number; PAI-1 = plasminogen activator inhibitor-1; PT = prothrombin time; TAFI = thrombin activatable fibrinolysis inhibitor; TATc = thrombin-antithrombin complex; ULN = upper limit of normal.

Source: CL102#1#, PC104#1# (F1K-MC-EVAD Clinical Study Report).

7.2. Effect of Drotrecogin Alfa (Activated) Administration on Biomarkers Over Time

For each biomarker, all patients with both baseline and at least one postbaseline measurement for Study Day 1 through Study Day 28 were included in the analyses. ANOVA based on ranked data was used to draw statistical conclusions between treatment groups. Within-group two-sided p-values testing the null hypothesis of no change from baseline were calculated using the Wilcoxon signed rank test for the change and percent change analyses. Ranked ANOVA was chosen as the primary basis for reporting p-values based on the skewness of the data distributions for many of the biomarkers. The LOCF method of imputation for missing observations was employed.

7.2.1. Analyses of Markers of Coagulation

Platelet Analyses. There were no statistically significant differences observed in percent change from baseline platelet counts between the two treatment groups.

APTT Analyses. Figure 7.1 shows the median percent change from baseline APTT on Study Days 1 through 7. The percent change in APTT from baseline was statistically significantly different between drotrecogin alfa (activated) patients and placebo patients on Study Days 1 through 4 (all $p<0.001$). The median APTT lengthened by approximately 7 seconds on Study Day 1 and subsequently shortened on Study Days 2 through 7 to below baseline levels in drotrecogin alfa (activated) patients. The magnitude of difference between the two treatment groups in median APTT ranged from 4.8 to 7.3 seconds. This finding is consistent with the anticoagulant pharmacodynamic effects of the molecule. At end of the 4-day study drug infusion, there were no statistically significant differences observed between treatment groups (on Study Days 5 through 7). The shortening of the APTT over time with drotrecogin alfa (activated) administration likely represents an improvement in the sepsis-associated coagulopathy and a decrease in the consumption of clotting factors associated with sepsis.

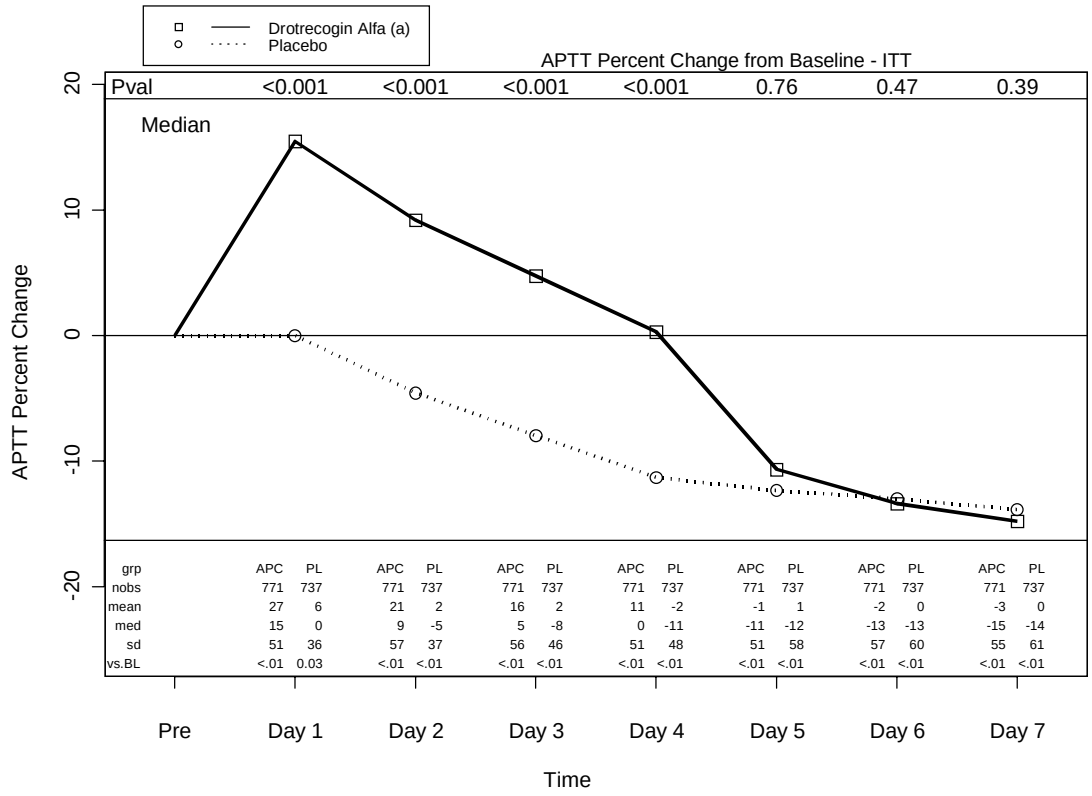


Figure 7.1. Percent change from baseline APTT on Study Days 1 through 7. Study F1K-MC-EVAD.

PT Analyses. The percent decrease in PT from baseline was less for drotrecogin alfa (activated) patients than for placebo patients on Study Day 1 ($p<0.001$) and on Study Days 2 and 4 ($p=0.018$ and $p=0.017$, respectively). The smaller decrease in PT in drotrecogin alfa (activated) patients over the 4-day study drug infusion period is consistent with the known anticoagulant pharmacodynamic effect of drotrecogin alfa (activated). The magnitude of the difference in median PTs between the two treatment groups was about 1 second. The effect of drotrecogin alfa (activated) on the PT was less than the effect on the APTT, consistent with the pharmacodynamic data in the Phase 1 and preclinical studies.

Protein C, Protein S and Antithrombin Activity Analyses. Figure 7.2 shows the median percent change from baseline Protein C activity levels on Study Days 1 through 7. The percent increases in Protein C activity levels from baseline were statistically significantly greater for drotrecogin alfa (activated) patients compared with placebo patients on Study Days 1 through 7 (all $p \leq 0.003$).

There were no statistically significant differences observed between the two treatment groups in the percent change from baseline Protein S activity levels.

Figure 7.3 shows the median percent change from baseline antithrombin activity levels on Study Days 1 through 7. There were no statistically significant differences observed between treatment groups in the percent change from baseline antithrombin activity levels through Study Day 4.

Protein C and antithrombin are endogenous regulators of coagulation and inflammation. In the setting of ongoing severe sepsis, inflammatory cytokines and thrombin directly damage the endothelial lining and activate the extrinsic pathway of coagulation, and these regulators (Protein C and antithrombin) are depleted due to consumption (utilization), degradation by neutrophil elastase, and decreased synthesis by the liver. If infusion of drotrecogin alfa (activated) is effective in turning off the interplay of inflammation and coagulation, Protein C and antithrombin levels should improve over time. Protein C levels increased from baseline over time in both treatment groups; however, the increase observed over Study Days 1 through 7 in drotrecogin alfa (activated) patients was statistically significantly greater than that in placebo patients. However, even with this increase, 57% of drotrecogin alfa (activated) survivors remained Protein C deficient at Study Day 4, suggesting that improvement in 28-day all-cause mortality with drotrecogin alfa (activated) administration is not dependent on returning endogenous Protein C levels to normal during study drug infusion.

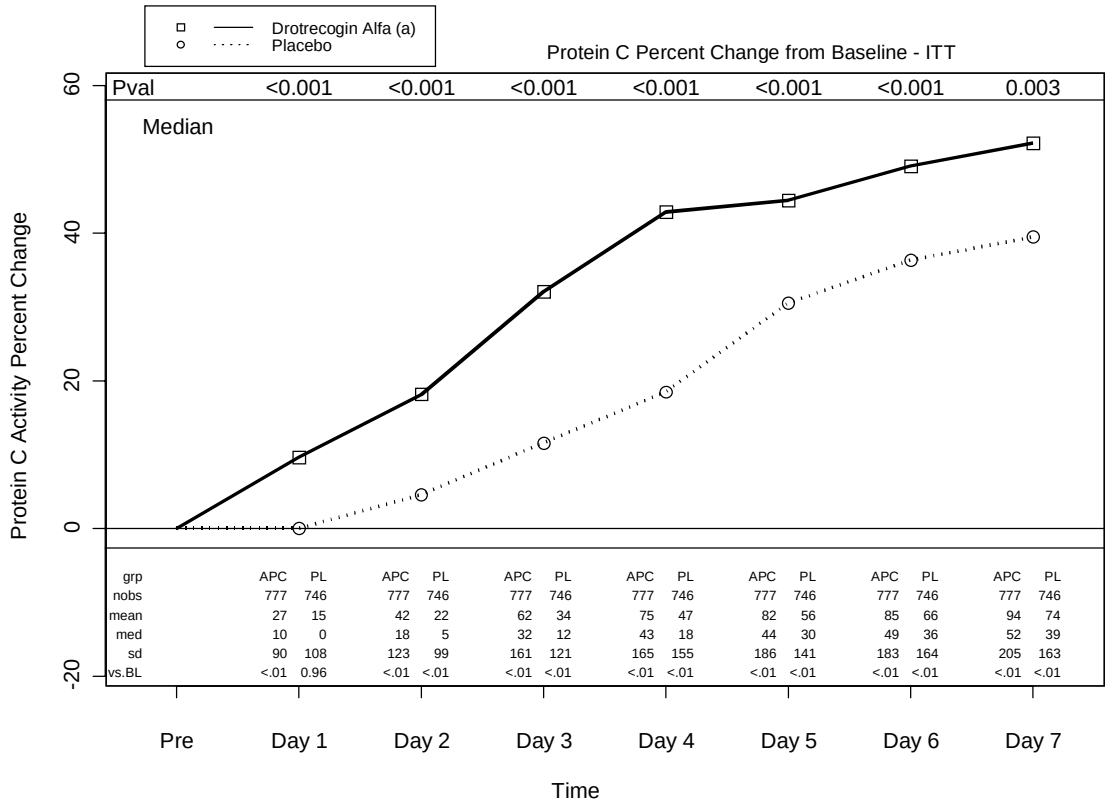


Figure 7.2. **Percent change from baseline Protein C activity levels on Study Days 1 through 7. Study F1K-MC-EVAD.**

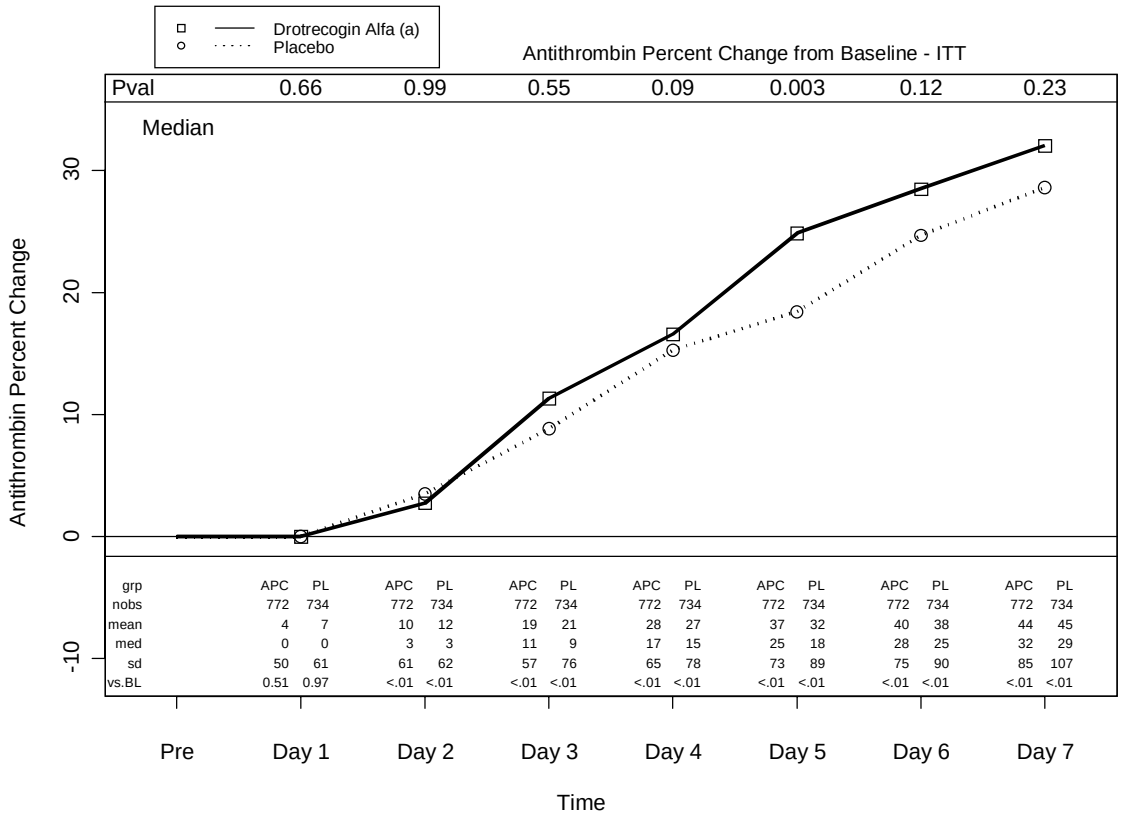


Figure 7.3. **Percent change from baseline antithrombin activity levels on Study Days 1 through 7. Study F1K-MC-EVAD.**

7.2.2. Analyses of Markers of Thrombin Generation

D-dimer Analyses. Figure 7.4 shows the median percent change from baseline D-dimer levels on Study Days 1 through 7. The percent decreases in D-dimer levels from baseline were statistically significantly greater for drotrecogin alfa (activated) patients compared with placebo patients on Study Days 1 through 7 (all p≤0.01).

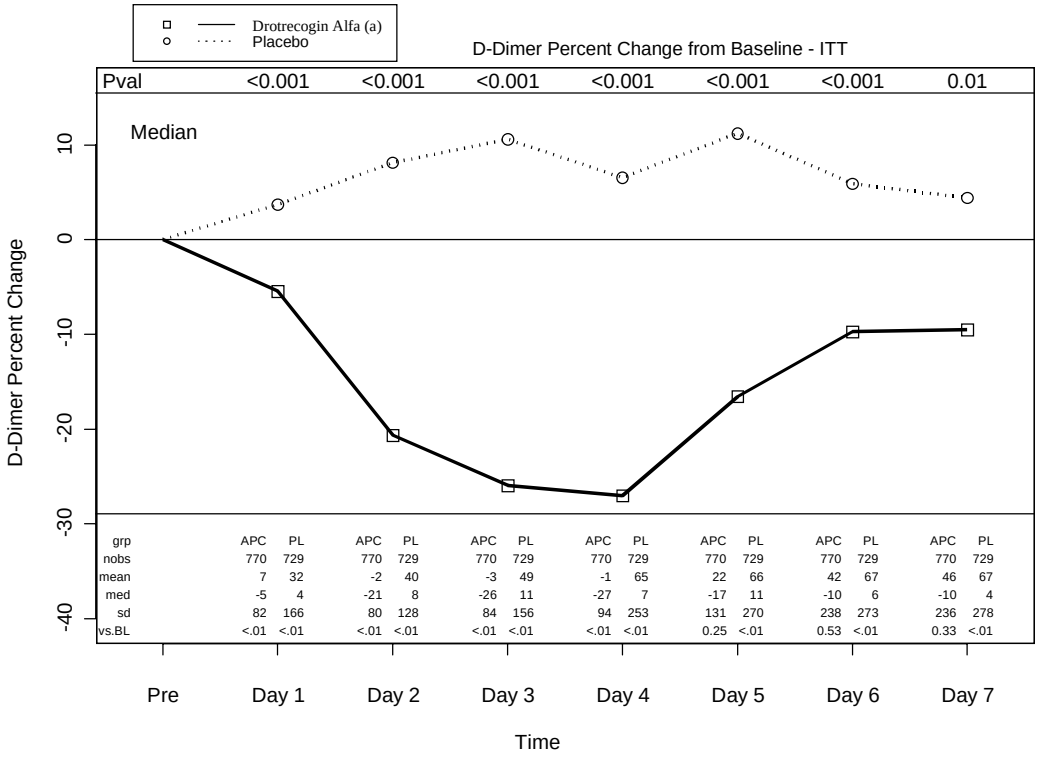


Figure 7.4. Percent change from baseline D-dimer levels on Study Days 1 through 7. Study F1K-MC-EVAD.

F1.2 Analyses. Figure 7.5 shows the median percent change from baseline F1.2 levels on Study Days 1, 2, 4, and 5. The percent decreases in F1.2 levels from baseline were statistically significantly greater for drotrecogin alfa (activated) patients compared with placebo patients on Study Days 1, 2, and 4 (all p<0.001).

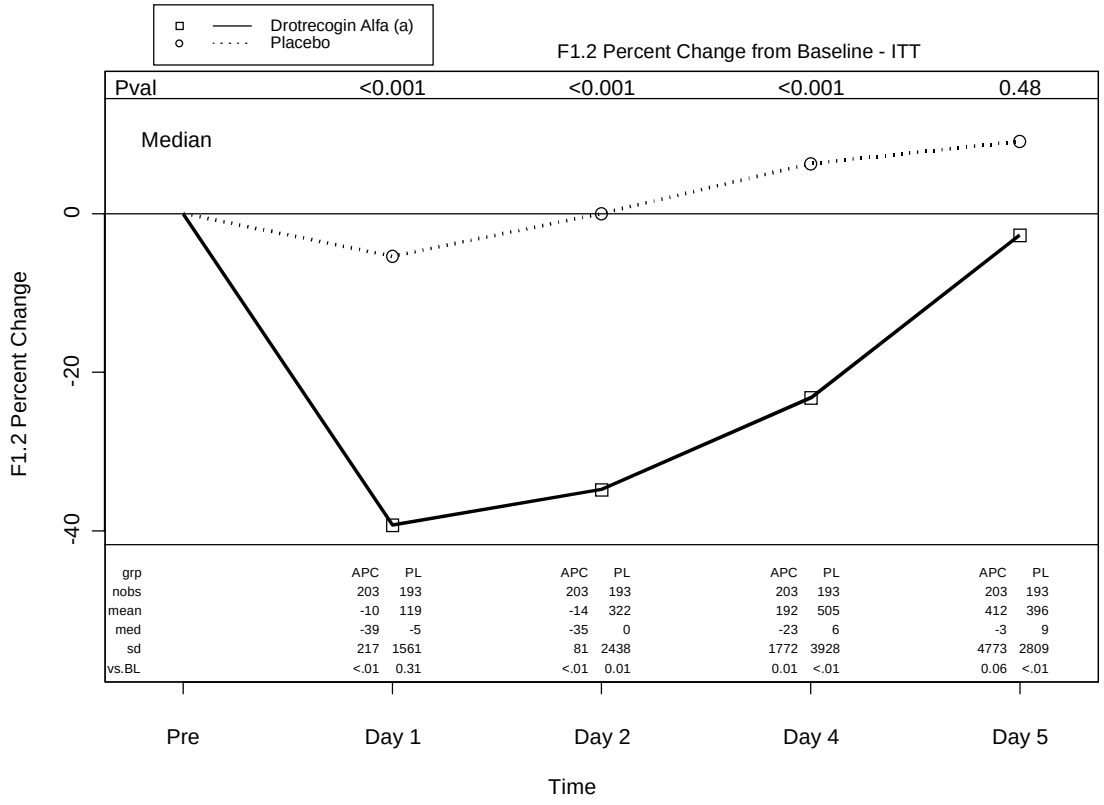


Figure 7.5. Percent change from baseline F1.2 levels on Study Days 1, 2, 4, and 5. Study F1K-MC-EVAD.

TATc Analyses. Figure 7.6 shows the median percent change from baseline TATc concentrations on Study Days 1, 2, 4, and 5. The percent decreases in TATc concentrations from baseline were statistically significantly greater for drotrecogin alfa (activated) patients compared with placebo patients on Study Days 1, 2, and 4 (all $p<0.001$).

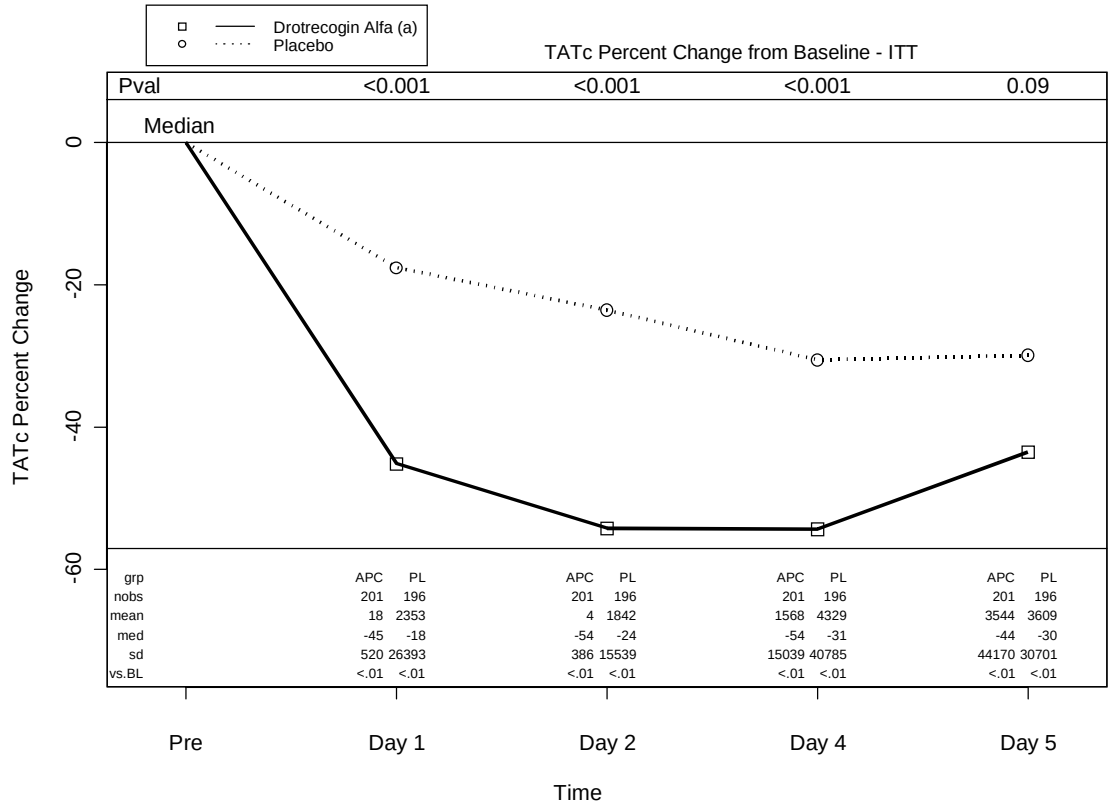


Figure 7.6. Percent change from baseline TATc concentrations on Study Days 1, 2, 4, and 5. Study F1K-MC-EVAD.

The markers of thrombin generation, D-dimer, F1.2, and TATc, all showed a more rapid decrease from baseline in the drotrecogin alfa (activated) patients compared with the placebo patients. These observations are indicative of the antithrombotic pharmacodynamic properties of the molecule. D-dimer levels rose following the end of the study drug infusion in drotrecogin alfa (activated) patients, but remained below baseline levels. This rise in D-dimer may reflect ongoing thrombin generation due to sepsis or the restoration of normal fibrinolysis, as D-dimer is also a product of fibrinolysis.

7.2.3. Analyses of Markers of Fibrinolysis

PAI-1 Analyses. Figure 7.7 shows the median percent change from baseline PAI-1 levels. There were no statistically significant differences observed in PAI-1 levels between the two treatment groups. However, the percent decreases in PAI-1 levels were greater for drotrecogin alfa (activated) patients on Study Days 1, 2, 4, and 5 compared with placebo patients; the differences between the two treatment groups approached statistical significance on Study Day 5 (p=0.053).

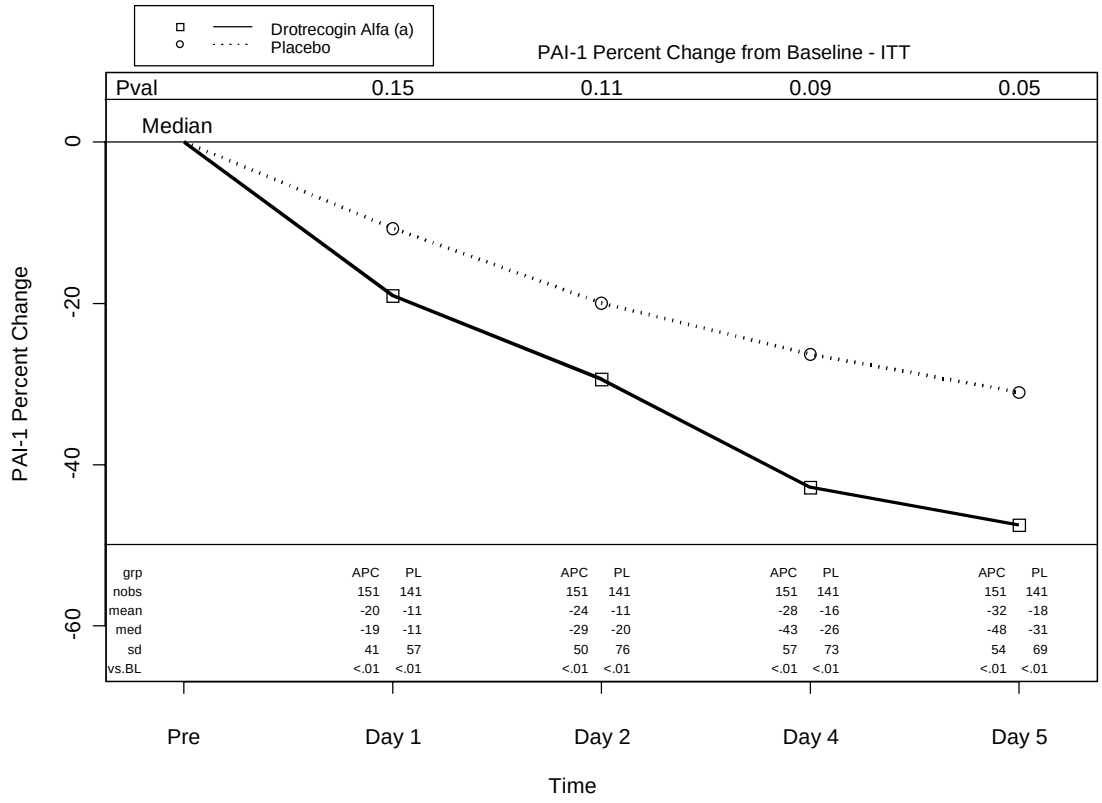


Figure 7.7. Percent change from baseline PAI-1 levels on Study Days 1, 2, 4, and 5. Study F1K-MC-EVAD.

TAFI Analyses. There were no statistically significant differences observed between treatment groups in the percent change from baseline TAFI levels.

α_2-Antiplasmin Analyses. There were no statistically significant differences observed between treatment groups in the percent change from baseline α_2-antiplasmin levels.

Plasminogen Analyses. Figure 7.8 shows the median percent change from baseline plasminogen levels for Study Days 1, 2, 4, and 5. There were no statistically significant differences observed between treatment groups in the percent change from baseline plasminogen levels for Study Days 1, 2, and 4. On Study Day 5, the percent increase

from baseline plasminogen levels were statistically significantly greater (p=0.023) for drotrecogin alfa (activated) patients compared with placebo patients.

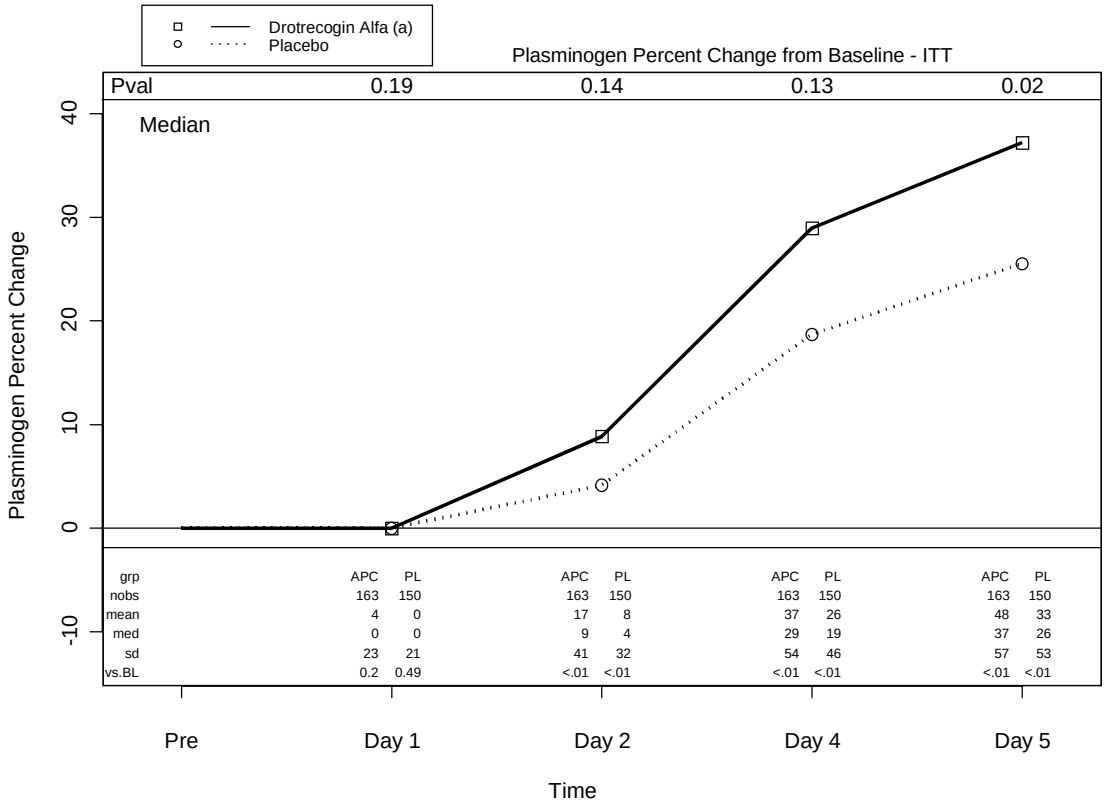


Figure 7.8. Percent change from baseline plasminogen levels on Study Days 1, 2, 4, and 5. Study F1K-MC-EVAD.

For markers of fibrinolysis, PAI-1, and plasminogen, PAI-1 levels decreased over Study Days 1 through 5 and plasminogen levels increased over Study Days 1 through 5. The increases in plasminogen levels and decreases in PAI-1 levels in the drotrecogin alfa (activated) treatment group may reflect a restoration of the fibrinolytic system. The decrease in thrombin generation with drotrecogin alfa (activated) administration may allow for an improvement in plasminogen levels. The effects observed on these markers of fibrinolysis in the drotrecogin alfa (activated) group suggest that drotrecogin alfa (activated) treatment helps restore the fibrinolytic potential in the treated patients.

7.2.4. Analyses of a Marker of Inflammation

IL-6 Analyses. Figure 7.9 shows the median percent change from baseline IL-6 levels on Study Days 1 through 7. The percent decreases in IL-6 levels from baseline were statistically significantly greater for drotrecogin alfa (activated) patients compared with placebo patients on Study Day 1, 2, 5, and 6 (all $p \leq 0.047$).

IL-6 was frequently used as a global inflammatory biomarker in clinical trials in severe sepsis. Several sepsis clinical trials were conducted with antagonists to TNF-α, including Etanercept, a drug approved for the treatment of rheumatoid arthritis. Three of these trials, including one testing Etanercept, did not demonstrate any difference in the IL-6 levels between active drug and placebo patients (Fisher et al. 1996; Clark et al. 1998; Abraham et al. 2001). Three other sepsis clinical trials of TNF-α antagonists reported significantly more rapid decrease in IL-6 levels (as change from baseline) in the treatment group compared to the placebo group. This more rapid decrease in IL-6 levels was demonstrated only at one time point of 8 or 24 hours (Fisher et al. 1993; Reinhart et al. 1996; Panacek et al. 2000). Comparatively, drotrecogin alfa (activated) treated patients in Study F1K-MC-EVAD had significantly more rapid reduction in IL-6 (as change from baseline) on multiple days, Day 1 ($p=0.01$) and on Days 4 through 7 ($p=0.02$), as shown in Figure 7.10. This more rapid decrease is indicative of the anti-inflammatory pharmacodynamic properties of the molecule.

The mechanism for the anti-inflammatory activity of drotrecogin alfa (activated) observed could be due to the indirect effect of limiting thrombin generation, as thrombin is known to have multiple proinflammatory properties in addition to its procoagulant activities. In addition, in vitro studies have suggested a potential direct anti-inflammatory effect of drotrecogin alfa (activated) through the modulation of cytokine production by mononuclear leukocytes and endothelial cells (Lorant et al. 1991, Palabrica et al. 1992; Lorant et al. 1993; Coughlin 1994; Xu and Esmon 1999; White et al. 2000; Joyce et al. 2001).

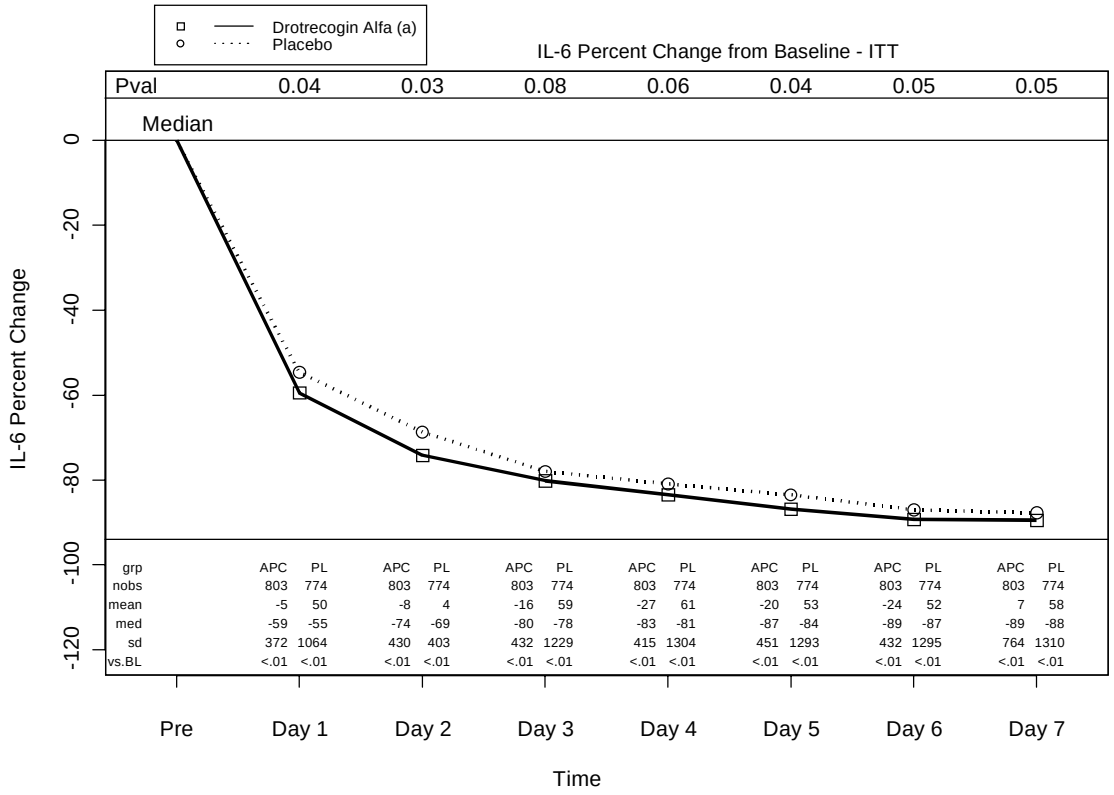


Figure 7.9. **Percent change from baseline IL-6 levels on Study Days 1 through 7. Study F1K-MC-EVAD.**

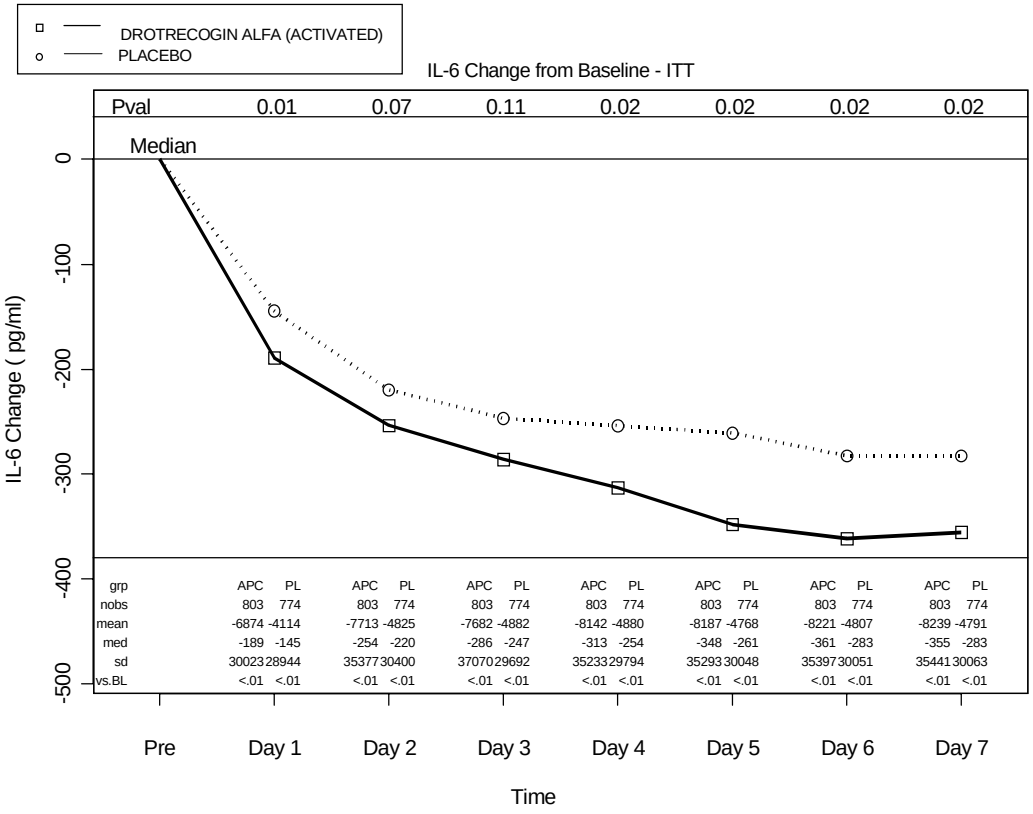


Figure 7.10. Change from baseline IL-6 levels on Study Days 1 through 7. Study F1K-MC-EVAD.

7.3. Pharmacodynamic Conclusions

- At study entry, almost all patients had generalized procoagulant and inflammatory responses to infection.
- The statistically significant prolongation of the APTT in the drotrecogin alfa (activated) patients as compared to the placebo patients during the 4-day study drug infusion is indicative of the anticoagulant pharmacodynamic effect of the drug.
- Drotrecogin alfa (activated) exerted an antithrombotic effect by limiting thrombin generation and improving the sepsis-associated coagulopathy, as shown by a more rapid improvement in markers of coagulation and fibrinolysis. Drotrecogin alfa (activated) caused a more rapid decline in thrombotic markers such as D-dimer, prothrombin F1.2, and thrombin-antithrombin levels and a more rapid increase in Protein C and antithrombin levels.

- Drotrecogin alfa (activated) restored fibrinolysis potential, as evidenced by a more rapid trend toward normalization in plasminogen and D-dimer levels and a trend toward more rapid decrease in PAI-1 levels.
- Patients with severe sepsis treated with drotrecogin alfa (activated) had a more rapid decline in IL-6 levels, a global marker of inflammation.

8. Safety Summary

This safety summary focuses primarily on data from the Phase 3 study F1K-MC-EVAD. In this study, analyses comparing the frequency of adverse events between the drotrecogin alfa (activated) treatment group with the placebo treatment group are confounded by the improved survival of patients in the drotrecogin alfa (activated) treatment group allowing for more follow-up time for adverse events to occur in these patients. No adjustment for this follow-up imbalance has been undertaken in the analyses presented.

8.1. Deaths

A statistically significant reduction in 28-day all-cause mortality was observed in drotrecogin alfa (activated) patients (24.7%) compared with placebo patients (30.8%; nonstratified p=0.0049).

The patient summaries of all patients who died in Study F1K-MC-EVAD were reviewed in a blinded manner by the sponsor's clinical research physicians and the event leading to death was adjudicated for all deaths. The majority of deaths in both treatment groups were due to either sepsis-induced multisystem organ failure or refractory septic shock (Table 8.1). Drotrecogin alfa (activated) patients did not experience more myocardial infarctions, primary cardiac arrhythmias, or cerebral infarcts leading to death. The drotrecogin alfa (activated) treatment group had more deaths due to hemorrhage, as assessed by the sponsor's clinical research physicians, compared with placebo patients [6 drotrecogin alfa (activated) patients versus 2 placebo patients].

Table 8.1. Summary of Cause of Death for All Deaths
Primary Analysis Population
Study F1K-MC-EVAD

Cause of Death Category	Drotrecogin Alfa (Activated)	Placebo	Total
Sepsis-Induced Multi-Organ Failure	96	102	198
Refractory Septic Shock	46	63	109
Respiratory Failure	28	46	74
Myocardial Infarction	9	11	20
Primary Cardiac Arrhythmia	6	9	15
Hemorrhage			
Cerebral	2	1	3
Pulmonary	2	0	2
Chest Trauma	1	0	1
Retroperitoneal	1	0	1
Thoracic	0	1	1
Other			
Cancer	3	4	7
Cardiogenic Shock	5	1	6
Cerebral Edema	3	1	4
Encephalopathy	2	2	4
Cerebral Infarction	0	3	3
Cardiomyopathy	2	0	2
Aortic Valve Endocarditis	0	1	1
Central Nervous System Event	0	1	1
Cerebral Arterial Thrombosis	0	1	1
Cerebral Embolism	0	1	1
Cerebral Herniation	1	0	1
Congestive Heart Failure	0	1	1
Hypoxic Brain Injury	0	1	1
Ischemic Bowel	0	1	1
Large and Small Bowel Infarction	0	1	1
Malignant Hyperthermia	0	1	1
Mitral Valve Rupture Secondary to Endocarditis	0	1	1
Pulmonary Embolism	1	0	1
Renal Failure	0	1	1
Tracheoesophageal Fistula	0	1	1
Unknown	2	3	5
Total	210	259	469

Source: Clintrace and Clinical Report Form Comments.

Of the six deaths assessed to be due to hemorrhage in drotrecogin alfa (activated) patients, four were considered study-drug related by the investigators and are discussed

below (Section 8.1.1). The remaining hemorrhagic deaths, two in drotrecogin alfa (activated) patients and two in placebo patients, were not considered by the investigator to be related to the bleeding event. These four deaths occurred in the following patients:

Placebo:

A 62-year-old patient experienced a fatal bleed after a thoracentesis 2 weeks following the completion of the study drug infusion. The investigator reported refractory septic shock as the cause of death.

A 46-year-old patient experienced a massive intracerebral bleed 6 days following study drug infusion. The investigator reported sepsis as the cause of death.

Drotrecogin alfa (activated):

A 39-year-old patient experienced a pulmonary hemorrhage 6 days after completion of the study drug infusion and died of refractory shock 2 days later.

A 69-year-old patient experienced a retroperitoneal bleed that was possibly related to drotrecogin alfa (activated) during the study drug infusion and died the next day of septic shock.

8.1.1. Deaths Considered Possibly Related to Study Drug

Of the 469 deaths observed in Study F1K-MC-EVAD [210 drotrecogin alfa (activated) patients and 259 placebo patients], six were considered possibly related to study-drug by the investigators [5 drotrecogin alfa (activated) patients and 1 placebo patient]. Of the five deaths in drotrecogin alfa (activated) patients, four were associated with serious bleeding events. The six deaths are described below.

Placebo:

A 39-year-old patient developed cerebral infarcts involving the right and left middle cerebral artery territories 35 hours following the completion of the placebo infusion. The patient died approximately 18 hours later.

Drotrecogin alfa (activated):

A 45-year-old patient experienced cerebral edema diagnosed 10 days after the completion of the drotrecogin alfa (activated) infusion. The patient died 2 days later. The patient had severe hypoxia during the course of her illness, which had required treatment with an extracorporeal membrane oxygenator (ECMO).

A 76-year-old patient experienced a fatal pulmonary hemorrhage 1 day into the drotrecogin alfa (activated) infusion. The bleeding event occurred in the presence of a profound coagulopathy with the APTT >150 seconds, a PT-INR of 3.7, and a platelet count of 19 GI/L. The patient did not have a history of any mass lesions of the lung and an autopsy was not performed.

A 74-year-old patient experienced a fatal cerebral hemorrhage diagnosed 14 hours into the drotrecogin alfa (activated) infusion. This event occurred in the setting of gram-negative sepsis with severe disseminated intravascular coagulation (DIC). The patient had an APTT of 49.2 seconds and a platelet count of 18 GI/L at the time of the event. The patient died approximately 5 hours after study drug discontinuation.

A 32-year-old patient suffered a fatal bleed as a result of severe trauma. The bleeding event was diagnosed 2 hours following completion of the 96 hour study drug infusion. The patient died approximately 4 hours after the completion of the drotrecogin alfa (activated) infusion. The patient sustained left pulmonary contusion, flail chest, splenic fracture, and an acetabular fracture as a result of a motor vehicle accident 3 days prior to study entry.

A 56-year-old patient experienced a fatal cerebral hemorrhage diagnosed 84 hours into the drotrecogin alfa (activated) infusion. During the infusion, the patient developed severe DIC with an APTT of 122 seconds and platelet count of 27 GI/L.

8.2. Adverse Event Analyses

Adverse events for Study F1K-MC-EVAD were analyzed in a treatment-emergent manner. Treatment-emergent adverse events are those events that occurred or worsened (if present at baseline) after the start of study drug administration. Since drotrecogin alfa (activated) may have antithrombotic and profibrinolytic properties, adverse events that were also considered bleeding events were assessed as a subset of all adverse events.

Treatment-emergent adverse events and serious adverse events were reported through Study Day 28. The treatment-emergent adverse events and serious adverse events that first occurred or were ongoing during the study drug infusion period were also assessed as a subset of all events occurring during the 28-day study period. The study drug infusion period for each patient was defined as the date of initiation of study drug administration to the date of last study drug discontinuation plus the next calendar day.

An event was classified as a treatment-emergent adverse event during the study drug infusion period if the following occurred: (1) the event was a new event with onset during the study drug infusion period and the event onset was on or before Study Day 6, or (2) the event was a preexisting condition (ie, ongoing at the start of study drug infusion) that worsened in severity on or before Study Day 6.

An event was classified as a serious adverse event during the study drug infusion period if the following occurred: (1) the event was a new event with onset during the study drug infusion period, the event onset was on or before Study Day 6, and the event became serious at any time during the 28-day study period, or (2) the event was a preexisting condition (ie, ongoing at the start of study drug infusion) that became serious at any time during the 28-day study period.

8.3. Bleeding Events

In Study F1K-MC-EVAD, patients who may have been at increased risk of bleeding were excluded from participation. The following exclusion criteria were related to bleeding risks:

- Any major surgery within 12 hours before study drug infusion, evidence of active bleeding postoperatively, or planned or anticipated surgery during the infusion period.
- A history of severe head trauma that required hospitalization, intracranial surgery, or stroke within 3 months of study entry.
- A history of intracerebral arteriovenous malformation, cerebral aneurysm, or central nervous system mass lesion.
- An epidural catheter or patients who anticipated receiving an epidural catheter during the infusion period.
- A history of congenital bleeding diatheses.
- Clinically significant gastrointestinal bleeding within 6 weeks of study entry that required medical intervention.
- Trauma patients at increased risk of bleeding.
- Known esophageal varices, chronic jaundice, cirrhosis, or chronic ascites.

In addition, patients recently treated with heparin (therapeutic doses of unfractionated heparin or low molecular weight heparins), warfarin, antiplatelets, thrombolytics, glycoprotein IIb/IIIa receptor antagonists, and antithrombin were excluded from participation in the study. Importantly, administration of prophylactic doses of unfractionated or low-molecular weight heparin was permissible.

Bleeding events defined as serious adverse events. Bleeding events reported as serious adverse events included the following: (a) any intracranial hemorrhage, (b) any life-threatening bleed, (c) a requirement of ≥3 units of packed red blood cells per day for 2 consecutive days, or (d) an event that met other criteria of serious adverse event definition.

8.3.1. Bleeding Events Reported as Serious Adverse Events

The drotrecogin alfa (activated) treatment group had a greater percentage of patients who experienced at least one bleeding event reported as a serious adverse event during the study drug infusion period (2.4% versus 1.0%; p=0.024) and during the 28-day study period (3.5% versus 2.0%; p=0.060) compared with the placebo treatment group (Table 8.2 and Table 8.3). The difference in the incidence of serious bleeding events between the two treatment groups occurred primarily during the study drug infusion period. The increased proportion of patients with at least one serious bleeding event in

the drotrecogin alfa (activated) treatment group was primarily related to traumatic injury or instrumentation of a major blood vessel or highly vascular organ.

Table 8.2. Summary of Serious Bleeding Events by Treatment Group and Site of Hemorrhage Primary Analysis Population Study Drug Infusion Period Study F1K-MC-EVAD

Site of Hemorrhage	Drotrecogin Alfa (Activated) N=850 Events (Proc-Rel)	Placebo N=840 Events (Proc-Rel)	Total N=1690
Gastrointestinal	5 (1)	4 (0)	9 (1)
Intra-abdominal	2 (2)	3 (0)	5 (2)
Intra-thoracic	4 (2)	0	4 (2)
Retroperitoneal	3 (3)	0	3 (3)
Cerebral Hemorrhage	2 (0)	0	2 (0)
Hemorrhage with an unidentified site of bleeding[a]	1 (0)	1 (0)	2 (0)
Genitourinary	2 (1)	0	2 (1)
Skin/soft tissue	1 (1)	0	1 (1)
Total	20 (10)	8 (0)	28 (10)
Percent	2.4%	1.0%	1.7%

Abbreviations: N = number of patients in treatment group; Proc-Rel = procedure-related.

[a] An event that required the administration of ≥3 units of packed red blood cells per day for 2 consecutive days, but a site of bleeding could not be identified.

Source: Clintrace.

Table 8.3. Summary of Serious Bleeding Events by Site of Hemorrhage and Treatment Group Primary Analysis Population 28-Day Study Period Study F1K-MC-EVAD

Body System	Drotrecogin Alfa (Activated) N=850 Events (Proc-Rel)	Placebo N=840 Events (Proc-Rel)	Total N=1690 Events (Proc-Rel)
Gastrointestinal	9 (1)	9 (0)	18 (1)
Intra-abdominal	3 (3)	4 (0)	7 (3)
Intrathoracic	6 (3)	1 (1)	7 (7)
Retroperitoneal	4 (4)	0	4 (4)
Cerebral Hemorrhage	2 (0)	1 (0)	3 (0)
Hemorrhage with an unidentified site of bleeding[a]	2 (2)	2 (2)	4 (4)
Genitourinary[a]	2 (1)	0	2 (1)
Skin/soft tissue	2 (1)	0	2 (1)
Total	30 (15)	17 (3)	47 (18)
Percent	3.5%	2.0%	2.8%

Abbreviations: N = number of patients in treatment group; Proc-Rel = procedure-related.

[a] An event that required the administration of ≥3 units of packed red blood cells per day for 2 consecutive days, but a site of bleeding could not be identified.

Source: Clintrace.

Of the drotrecogin alfa (activated) patients that experienced a serious bleeding event, four patients died due to the serious bleeding event. A total of 3 patients with a fatal bleeding event (2 patients with intracranial hemorrhages and 1 patient with a pulmonary hemorrhage) had profound coagulopathy and severe thrombocytopenia (<30 GI/L). The fourth fatal bleeding event was an intrathoracic bleed from severe trauma that occurred after completion of the 96 hour study drug infusion period. The nonfatal serious bleeding events associated with drotrecogin alfa (activated) administration primarily occurred in patients with gastrointestinal ulceration, injury to a blood vessel, or following instrumentation or traumatic injury to a highly vascular organ such as the kidney or lung.

8.3.2. Bleeding Events Reported as Treatment-Emergent Adverse Events

Bleeding events reported as treatment-emergent adverse events were common in both treatment groups. However, a statistically significantly greater proportion of drotrecogin alfa (activated) patients experienced at least one bleeding event reported as a treatment-emergent adverse event during the study drug infusion period (18.8% versus 10.8%;

p<0.001) and during the 28-day study period (24.9% versus 17.7%; p<0.001). Similar to the serious bleeding events, the differences in the incidence of treatment-emergent bleeding events between the two treatment groups occurred primarily during the study drug infusion period. Cutaneous (injection site hemorrhage and ecchymosis) and gastrointestinal tract bleeding (gastrointestinal hemorrhage, melena, and rectal hemorrhage) were the most commonly reported bleeding events.

The majority of bleeding events were mild in severity; however, the drotrecogin alfa (activated) treatment group had a higher proportion of patients compared with the placebo treatment group who experienced a mild (10.5% versus 7.7%), moderate (5.5% versus 1.8%), or severe (2.8% versus 1.3%) bleeding event during the study drug infusion period (Figure 8.1). This pattern continued through the 28-day study period (Figure 8.2).

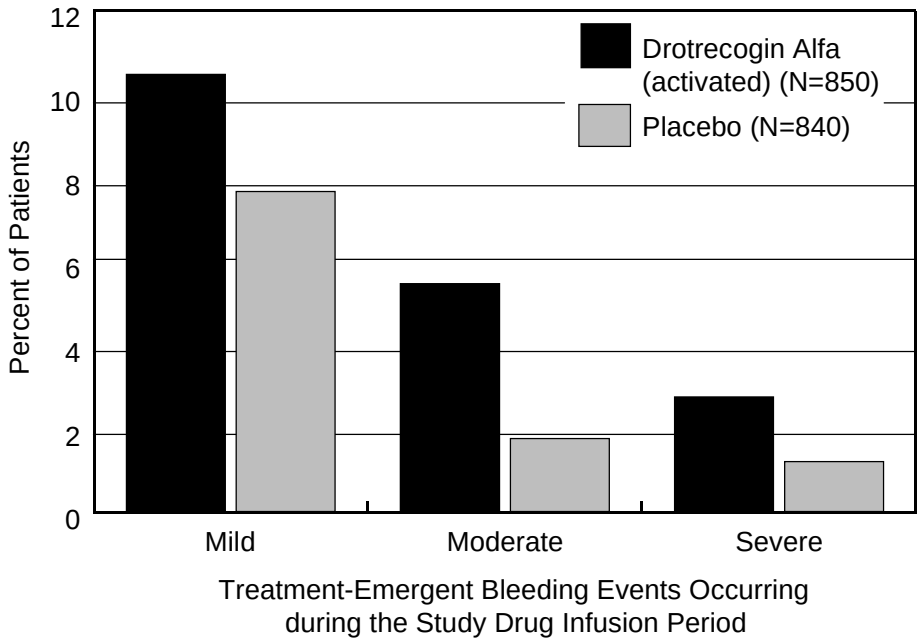


Figure 8.1. **Percentage of patients who experienced a bleeding event reported as a treatment-emergent adverse event during the study drug infusion period by maximum severity. Study F1K-MC-EVAD.**

Patients who had more than one bleeding event were counted only once according to the event with the most severe classification.

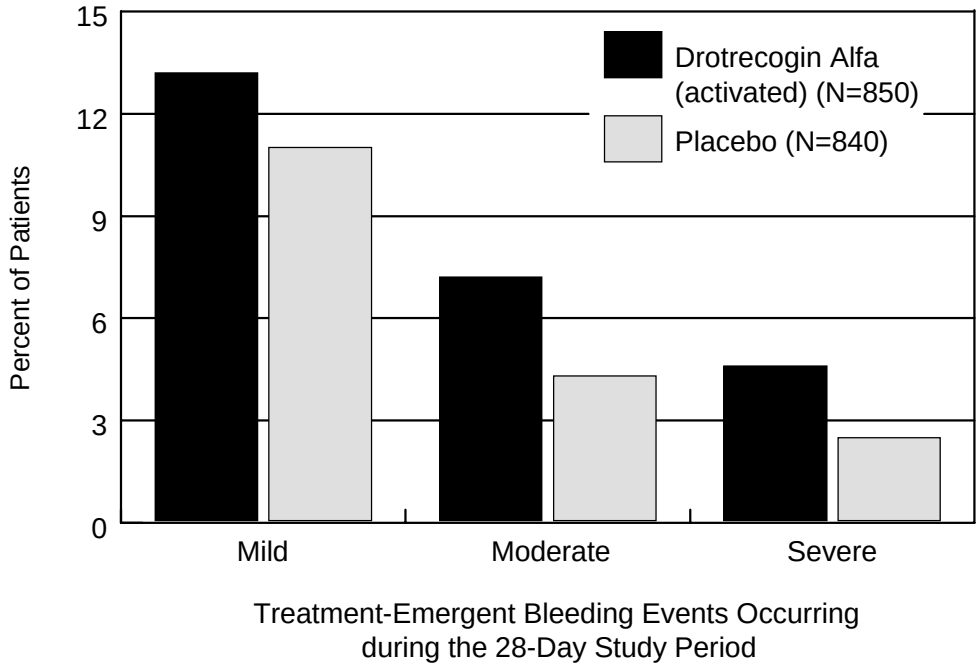


Figure 8.2. **Percentage of patients who experienced a bleeding event reported as a treatment-emergent adverse event during the 28-day study period by maximum severity. Study F1K-MC-EVAD.**

Patients who had more than one bleeding event were counted only once according to the event with the most severe classification.

8.4. Adverse Events Excluding Bleeding Events

Given the nature of severe sepsis, multiple adverse events would be expected to occur during the study. Because many of these adverse events were also related to efficacy measures, those events related to organ failure and SIRS criteria were not reported as adverse events or serious adverse events unless they were thought by the investigator to be causally related to the study drug.

Treatment-Emergent Adverse Events. Adverse events were common in both treatment groups. There was no statistically significant difference between the drotrecogin alfa (activated) treatment group compared with the placebo treatment group in the proportion of patients who experienced at least one treatment-emergent adverse event during the study drug infusion period (68.6% versus 65.0%) or during the 28-day study period (81.8% versus 77.7%). Statistically significant differences between the two treatment

groups in the proportion of patients who experienced a specific type of adverse event were infrequent. No clinical correlation could be drawn from the nonbleeding adverse events that occurred statistically significantly more often in either treatment group.

Serious Adverse Events. There was no statistically significant difference between the drotrecogin alfa (activated) treatment group and the placebo treatment group in the percentage of patients who experienced at least one serious adverse event during the study drug infusion period (6.8% versus 6.5%) and during the 28-day study period (12.5% versus 12.1%). There were also no serious adverse events, other than the bleeding event of hemorrhage, individually or grouped by body system, for which differences between the treatment groups were statistically significant during the study drug infusion period and during the 28-day study period. Importantly, the drotrecogin alfa (activated) group experienced fewer cardiac dysrhythmias (heart arrest, ventricular tachycardia, atrial fibrillation, arrhythmia, supraventricular tachycardia, ventricular fibrillation, and ventricular arrhythmia) reported as serious adverse events than did the placebo group during the 28-day study period (2.0% versus 3.3%, respectively; p=0.089). Table 8.4 contains a summary of the serious adverse events experienced by more than 2 patients during the 28-day study period.

Table 8.4. Summary of Serious Adverse Events Experienced by Greater Than 2 Patients 28-Day Study Period Study F1K-MC-EVAD

Event Classification	Drotrecogin Alfa (Activated) (N=850) n (%)	Placebo (N=840) n (%)	Total (N=1690) n (%)	p-Value
Patients with ≥1 Event	106 (12.5)	102 (12.1)	208 (12.3)	0.838
Heart Arrest	11 (1.3)	11 (1.3)	22 (1.3)	0.978
Myocardial Infarct	8 (0.9)	10 (1.2)	18 (1.1)	0.618
Gastrointestinal Hemorrhage	6 (0.7)	8 (1.0)	14 (0.8)	0.576
Hemorrhage	11 (1.3)	2 (0.2)	13 (0.8)	0.013
Cerebral Infarct	3 (0.4)	6 (0.7)	9 (0.5)	0.307
Pneumonia	4 (0.5)	3 (0.4)	7 (0.4)	0.717
Pulmonary Embolus	2 (0.2)	5 (0.6)	7 (0.4)	0.249
Ventricular Tachycardia	3 (0.4)	4 (0.5)	7 (0.4)	0.693
Endocarditis	2 (0.2)	3 (0.4)	5 (0.3)	—
Sepsis	3 (0.4)	2 (0.2)	5 (0.3)	—
Ventricular Fibrillation	2 (0.2)	3 (0.4)	5 (0.3)	—
Atrial Fibrillation	1 (0.1)	3 (0.4)	4 (0.2)	—
Cardiovascular Disorder	2 (0.2)	2 (0.2)	4 (0.2)	—
Encephalopathy	1 (0.1)	3 (0.4)	4 (0.2)	—
Supraventricular Tachycardia	1 (0.1)	3 (0.4)	4 (0.2)	—
Arrhythmia	0	3 (0.4)	3 (0.2)	—
Carcinoma	1 (0.1)	2 (0.2)	3 (0.2)	—
Cerebral Hemorrhage	2 (0.2)	1 (0.1)	3 (0.2)	—
Dyspnea	0	3 (0.4)	3 (0.2)	—
Gastrointestinal Disorder	0	3 (0.4)	3 (0.2)	—
Healing Abnormal	2 (0.2)	1 (0.1)	3 (0.2)	—
Infection	1 (0.1)	2 (0.2)	3 (0.2)	—
Lung Hemorrhage	3 (0.4)	0	3 (0.2)	—
Pneumothorax	1 (0.1)	2 (0.2)	3 (0.2)	—

Source: AE111#1#

8.4.1. Incidence of Thrombotic Events

The incidence of thrombotic events in the drotrecogin alfa (activated) treatment group relative to the placebo group was of interest as it may be an indicator of the development of anti-Activated Protein C antibodies capable of neutralizing the activity of endogenous Activated Protein C.

The drotrecogin alfa (activated) treatment group experienced fewer thrombotic events (pulmonary, myocardial, cerebral, venous, or arterial thrombotic events) reported as serious adverse events during the 28-day study period compared with the placebo treatment group (2.0% versus 3.0%, respectively; p=0.197). This finding suggests that a hypercoagulable state does not occur following termination of the drotrecogin alfa (activated) infusion. These data are consistent with the absence of the development of neutralizing antibodies against Activated Protein C in patients receiving drotrecogin alfa (activated) (see Section 10 for a discussion of immunogenicity testing).

8.4.2. Risk of infection

In Study F1K-MC-EVAD, postbaseline culture data were analyzed. Positive cultures noted 48 hours after the initiation of study drug infusion through Study Day 28 were recorded and were assessed by the investigator to be either pathogen or contamination. In addition, where the positive culture was thought to be a pathogen, the investigator assessed whether this was a sequela of the original sepsis event or a new infection.

There was no statistically significant difference between the two treatment groups in the proportion of patients with at least one pathogen indicating a sequela of the infection that caused the original sepsis event or the proportion of patients with at least one pathogen indicating a new infection. The percentage of patients with a postbaseline culture indicating a sequela of the infection that caused the original sepsis event was 16.6% for drotrecogin alfa (activated) patients and 17.6% for placebo patients; the percentage with a new infection was 25.5% for the drotrecogin alfa (activated) patients and 25.1% for the placebo patients. These findings suggest that a predisposition to new infections does not occur following drotrecogin alfa (activated) administration.

8.5. Drug-Drug Interactions

Drotrecogin alfa (activated) has significant anticoagulant activity. Therefore, concomitant use of therapeutic concentrations of other anticoagulants and fibrinolytic agents were not allowed during Study F1K-MC-EVAD. Low dose heparin is commonly used in the intensive care setting to prevent the development of deep venous thrombosis and to maintain catheter patency; such doses of heparin were allowed during Study F1K-MC-EVAD. Analysis of drug-drug interaction was consequently limited to the potential interaction between low-dose heparin and drotrecogin alfa (activated).

Heparin Use. A patient was classified as being exposed to heparin if the patient received any dose of fractionated or low molecular weight heparin by any route (excluding the use

of heparin to maintain vascular catheter patency) during the study drug infusion or the next calendar day after the end of the infusion. Since heparin use is defined based on postbaseline results, caution must be used in drawing conclusions from these analyses. The observed mortality was similar for drotrecogin alfa (activated) patients who were exposed or not exposed to heparin: 158 of 634 (24.92%) patients exposed to heparin died versus 52 of 216 (24.07%) patients not exposed to heparin. In addition, the observed proportion of drotrecogin alfa (activated) patients who experienced a bleeding event reported as a serious adverse event during the study drug infusion period was similar regardless of whether the patient was exposed or not exposed to heparin (15 of 634 patients, 2.37% versus 5 of 216 patients, 2.31%).

8.6. Discontinuations of Study Drug Infusion Due to Adverse Events

A statistically significant higher proportion of drotrecogin alfa (activated) patients had the study drug infusion permanently discontinued due to an adverse event (6.4% versus 3.6%; p=0.009). Compared to placebo patients, a higher proportion of drotrecogin alfa (activated) patients had their study drug infusion permanently discontinued because of an adverse event related to bleeding [drotrecogin alfa (activated): 26/850, 3.1%; placebo: 10/840, 1.2%]. Gastrointestinal hemorrhage was the most commonly reported adverse event that resulted in permanent discontinuation of the study drug infusion in the drotrecogin alfa (activated) and placebo treatment groups (1.3% and 0.6%, respectively; p=0.138). The drotrecogin alfa (activated) treatment group had a statistically significantly higher proportion of patients with myocardial infarct reported as the reason for permanently discontinuing study drug infusion (0.7% versus 0.0%). However, there were no significant differences in the proportion of patients reporting myocardial infarcts as a serious adverse event [drotrecogin alfa (activated): 0.9%; placebo: 1.2%] or treatment-emergent adverse event during the study drug infusion period [drotrecogin alfa (activated): 14 patients, 1.6%; placebo: 12 patients, 1.4%] or during the 28-day study period [16 patients, 1.9% for both drotrecogin alfa (activated) and placebo patients]. Table 8.5 contains a summary of adverse events that led to discontinuation of the study drug infusion.

For both treatment groups, the source of the gastrointestinal tract bleeding was predominantly intestinal ulcerations identified during diagnostic procedures.

Table 8.5. Summary of Adverse Events Leading to Discontinuation of Study Drug Infusion in More Than 1 Patient Study F1K-MC-EVAD

Event Classification	Drotrecogin Alfa (Activated) (N=850) n (%)	Placebo (N=840) n (%)	Total (N=1690) n (%)	p-Value
Patients Discontinuing	54 (6.4)	30 (3.6)	84 (5.0)	0.009
Gastrointestinal Hemorrhage	11 (1.3)	5 (0.6)	16 (0.9)	0.138
Myocardial Infarct	6 (0.7)	0	6 (0.4)	0.015
Hemorrhage	5 (0.6)	2 (0.2)	7 (0.4)	0.262
Coagulation Disorder	4 (0.5)	0	4 (0.2)	—
Pulmonary Embolus	3 (0.4)	3 (0.4)	6 (0.4)	0.988
Hematuria	2 (0.2)	0	2 (0.1)	—
Thrombocytopenia	2 (0.2)	2 (0.2)	4 (0.2)	—
Lung Hemorrhage	2 (0.2)	0	2 (0.1)	—
Injection Site Reaction	2 (0.2)	0	2 (0.1)	—
Anemia	2 (0.2)	1 (0.1)	3 (0.2)	—
Cerebral Hemorrhage	2 (0.2)	0	2 (0.1)	—
Heart Arrest	1 (0.1)	2 (0.2)	3 (0.2)	—
Deep Thrombophlebitis	1 (0.1)	1 (0.1)	2 (0.1)	—
Ventricular Tachycardia	1 (0.1)	1 (0.1)	2 (0.1)	—
Rupture of Spleen	0	2 (0.2)	2 (0.1)	—
Thrombosis	0	2 (0.2)	2 (0.1)	—

Source: AE558#1#.

8.7. Safety Analyses by Average-Observed Steady-State Concentration

Drotrecogin alfa (activated) patients with available pharmacokinetic data (326 patients) were segmented into quartiles based on their average-observed steady-state Activated Protein C concentration. These quartiles corresponded to the following values:

- First quartile, 14.1 ng/mL to 34.9 ng/mL
- Second quartile, 35.1 ng/mL to 44.9 ng/mL
- Third quartile, 45.1 ng/mL to 62.0 ng/mL
- Fourth quartile, 62.1 ng/mL to 390.6 ng/mL (median = 82.6 ng/mL).

Table 8.6 contains a summary of the safety profile by Activated Protein C steady-state concentration quartile. There were no statistically significant differences observed among the average-observed steady-state Activated Protein C concentration quartiles with respect to the proportion of patients having at least one bleeding event reported as a serious adverse event that occurred during the study drug infusion period or during the 28-day study period, and the proportion of patients having at least one serious adverse event that occurred during the study drug infusion period or during the 28-day study period.

There was a statistically significant relationship between average-observed steady-state Activated Protein C concentration and 28-day all-cause mortality ($p=0.017$). Patients in the fourth quartile had the greatest mortality rate (33.3%). This mortality rate is similar to the overall placebo mortality rate. In addition, 9 of the 10 patients having the highest Activated Protein C concentrations (≥131.4 ng/mL) survived 28 days.

As all patients received the same infusion rate, the average-observed steady-state concentration directly reflects the clearance of Activated Protein C. Therefore, patients in the fourth concentration quartile would have had the lowest Activated Protein C clearance rates. As discussed in Section 6.3, patients who had lower clearance rates were more likely to be older (>65 years), have higher baseline APACHE II scores (in the fourth quartile), have higher baseline AST and ALT concentrations (>3 time ULN), have more abnormal renal function at baseline (Cockroft-Gault creatinine clearance of <20 mL/min), and have longer prothrombin times at baseline (>1.2 times ULN).

Given the absence of differences in the percent of patients experiencing a serious adverse event or a bleeding event reported as a serious adverse event between concentration quartiles, the finding of a higher mortality in the fourth concentration quartile does not represent a safety concern. The observed higher mortality in the fourth concentration quartile is most likely due to a patient selection bias.

Table 8.6. Summary of Safety Profile by Activated Protein C Steady-State Concentration Quartile Study F1K-MC-EVAD

	Steady-State Concentration Quartile					
	1st (N=81) n (%)	2nd (N=83) n (%)	3rd (N=81) n (%)	4th (N=81) n (%)	Total (N=326) n (%)	p-Value
28-Day Mortality Status						
Dead at Day 28	12 (14.8)	14 (16.9)	16 (19.8)	27 (33.3)	69 (21.2)	0.017
Bleeding Events Reported as Serious Adverse Events						
Infusion Period	2 (2.5)	0	1 (1.2)	2 (2.5)	5 (1.5)	0.516
28-Day Study Period	3 (3.7)	0	3 (3.7)	3 (3.7)	9 (2.8)	0.367
Serious Adverse Events						
Infusion Period	4 (4.9)	4 (4.8)	6 (7.4)	6 (7.4)	20 (6.1)	0.824
28-Day Study Period	7 (8.6)	10 (12.0)	15 (18.5)	13 (16.0)	45 (13.8)	0.274

Source: AE147#7#.

8.8. Analysis of Transfusion Data

Data were collected on the number of patients who received packed red blood cells, fresh frozen plasma, or platelets and the number of units received by each patient. There were no statistically significant differences between the treatment groups in the percentage of patients who received packed red blood cells and platelets or in the mean number of units received by each patient: 62.7% of drotrecogin alfa (activated) patients received packed red blood cells versus 58.3% of placebo patients; 13.4% of drotrecogin alfa (activated) patients received platelets versus 11.4% of placebo patients. A statistically significantly greater percentage of drotrecogin alfa (activated) patients received fresh frozen plasma compared with placebo patients (23.5% versus 19.3%; p=0.033). However, there was no difference in the mean number of units received by patients in each treatment group. After adjustment for the duration of survival, red blood cell transfusion requirements were similar in the two treatment groups (p=0.90).

8.9. Laboratory Analyses

In Study F1K-MC-EVAD, extensive analyses of the central chemistry and hematology data were conducted. Based on the assessments of the central laboratory data, there were no identifiable safety concerns associated with drotrecogin alfa (activated) administration.

8.10. Conclusions

- Serious bleeding events occurred in a higher percentage of patients in the drotrecogin alfa (activated) treatment group during the study drug infusion period and the 28-day study period compared with the placebo treatment group. The difference in the incidence of serious bleeding events occurred primarily during the study drug infusion period. Serious bleeding events were almost always associated with vessel trauma, tissue trauma (either accidental or iatrogenic), or ulceration of the gastrointestinal tract. [In future studies or clinical practice, avoidance of invasive procedures during the administration of drotrecogin alfa (activated) may reduce the potential risk of bleeding. In addition, visualization of the suspected site of bleeding should be considered for those patients who experience gastrointestinal tract bleeding associated with drotrecogin alfa (activated) administration, even if drotrecogin alfa (activated) administration is permanently discontinued.]
- The administration of drotrecogin alfa (activated) was not associated with an increased incidence of thrombotic events, indicating that a prothrombotic effect is not associated with the discontinuation of the drotrecogin alfa (activated) infusion.
- The administration of drotrecogin alfa (activated) was not associated with an increased incidence of new infections.
- The coadministration of prophylactic-dose heparin and drotrecogin alfa (activated) was not associated with an increased risk of serious bleeding.
- There were no other safety concerns associated with the administration of drotrecogin alfa (activated) to patients with severe sepsis.

9. Benefit - Risk Analysis

All new medications must be thoroughly evaluated in terms of safety and efficacy, and the benefits must be weighed against the risks. Therefore, an extensive analysis of benefit-risk was conducted for the population as a whole and for subpopulations of patients in Study F1K-MC-EVAD.

For this discussion, the benefit of drotrecogin alfa (activated) was defined as an improvement in survival associated with this therapy. The additional beneficial effect of drotrecogin alfa (activated) on morbidity was not included in this assessment, as it was deemed secondary to the unprecedented mortality benefit.

The only adverse event associated with drotrecogin alfa (activated) was bleeding. Since most bleeding events were mild in severity, the potential risk associated with the administration of drotrecogin alfa (activated) was considered to be the increased incidence of bleeding events reported as serious adverse events. In Study F1K-MC-EVAD, serious bleeding events were defined as: (a) any intracranial hemorrhage, (b) any life-threatening bleed, (c) any bleeding event requiring transfusion of three or more units of packed red blood cells per day on 2 consecutive days, or (d) any bleeding event meeting any of the other criteria defining a serious adverse event. Given that the benefit being assessed was defined as an improvement in survival, use of only serious bleeding events as opposed to all serious and non-serious bleeding events in the benefit-risk assessment is considered appropriate.

The need for red blood cell transfusion as a potential risk associated with drotrecogin alfa (activated) was also considered. However, a high percentage (58.3%) of placebo patients received at least 1 unit of packed red blood cells during the 28-day study period while only 17.7% of placebo patients experienced a serious or non-serious bleeding event reported as an adverse event during the same time period. In addition, the requirement for transfusion may be influenced by patient outcome (ie, patients who die early in their hospital course may not survive long enough to require a transfusion). Since the majority of patients receiving a transfusion did not experience a serious or non-serious bleeding event, transfusion was precluded from use as a potential risk associated with drotrecogin alfa (activated) in the benefit-risk analysis.

To avoid double counting patients who had serious bleeding events and died from any cause in a benefit-risk assessment, “risk” can be quantified by including only those serious bleeding events that occurred in survivors. Non-survivors who had serious bleeding events are already included in the benefit-risk assessment by reducing the observed survival benefit, even though they may not have died of a bleeding complication.

9.1. Benefit–Risk Analysis for the Entire Patient Population Study F1K-MC-EVAD

The results of Study F1K-MC-EVAD indicate that drotrecogin alfa (activated) substantially reduces mortality in patients with severe sepsis. For the entire population studied, the magnitude of the benefit was the 6.1% absolute mortality reduction observed with drotrecogin alfa (activated) compared to placebo.

The administration of drotrecogin alfa (activated) was also associated with an increase in the percentage of patients experiencing a serious bleeding event. In the entire patient population there were 47 patients who experienced a serious bleeding event: 17 patients in the placebo group (14 not procedure-related) and 30 patients in the drotrecogin alfa (activated) group (15 not procedure-related). Serious bleeding events frequently resulted from injury to a blood vessel (traumatic or iatrogenic) or following instrumentation of a highly vascular organ, such as the kidney or lung. In a blinded assessment of the primary cause of death, 2 placebo patients (0.2%) and 6 drotrecogin alfa (activated) patients (0.7%) died of bleeding complications.

Of the 47 serious bleeding events, 26 occurred in patients who survived the 28-day study period – 8 (0.95%) in the placebo group and 18 (2.12%) in the drotrecogin alfa (activated) group. Thus, the magnitude of the risk associated with therapy was a 1.2% absolute increase in serious bleeding events in surviving patients. Hence, for the population as a whole, the administration of drotrecogin alfa (activated) was associated with a high benefit to risk profile.

To explore the robustness of this conclusion and to account for the use of benefit and risk measures that are not similar, the net benefit for the entire patient population was assessed under conditions where the burden of an additional serious bleeding event was varied against the value of an additional life saved (Figure 9.1).

Net benefit in Figure 9.1 was calculated as follows:

$$\text{Net benefit} = \text{MARR} - (1/k) * \text{SBARI}$$

where:
MARR = Mortality Absolute Risk Reduction (Benefit)
SBARI = Serious Bleed Absolute Risk Increase for survivors (Risk)
k = Number of additional non-fatal serious bleeding events one would accept to save an additional life.

In this analysis, the number of additional serious bleeding events one would accept for each additional life saved was varied between 1 and 100. The net benefit associated with each value-burden relationship is displayed on the ordinate. A net benefit greater than 0 indicates a benefit associated with the administration of drotrecogin alfa (activated).

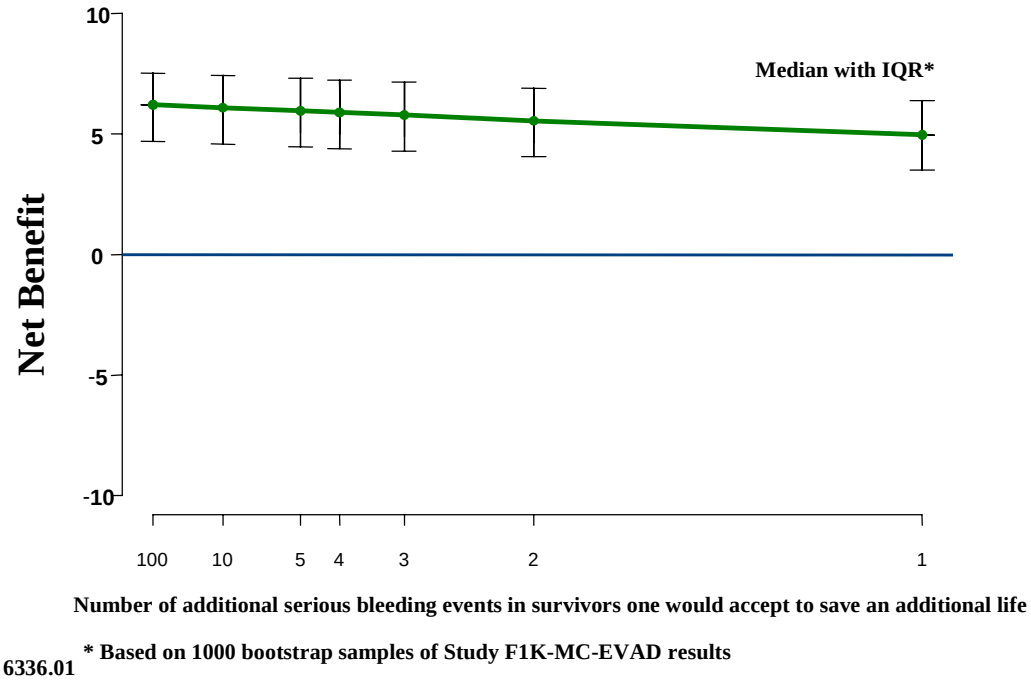


Figure 9.1. Benefit for entire patient population. Study F1K-MC-EVAD.

As depicted in Figure 9.1, regardless of the relationship placed on the risk of non-fatal serious bleeding versus the benefit of reduced mortality, the data from Study F1K-MC-EVAD demonstrate a high benefit to risk profile associated with the administration of drotrecogin alfa (activated).

9.2. Benefit-Risk Analysis for Subpopulations of Patients in Study F1K-MC-EVAD

Patients with severe sepsis enrolled in Study F1K-MC-EVAD represented a clinically heterogeneous population with respect to underlying co-morbidities, type of infection, site of infection, and associated types of organ dysfunction. Therefore, a number of subgroup analyses were performed to assess the consistency of the drotrecogin alfa (activated) treatment benefit and treatment risk across subpopulations of patients in Study F1K-MC-EVAD. These subgroup analyses allowed for exploration of potential drug-gender, drug-age, drug-ethnicity, and drug-disease interactions. As with all subgroup analyses, the results presented below must be interpreted with caution, bearing in mind the following caveats:

- Subgroup analyses are exploratory and hypothesis generating. As with almost all clinical trials, Study F1K-MC-EVAD was sized to detect a treatment effect for the overall primary analysis population only and not for subgroups. Importantly, even if there is a constant treatment benefit across a patient population, no trial can ensure definitive statistical evidence of a treatment benefit in all subgroups.
- The results of individual subgroup analyses must be interpreted in the context of the multiplicity of subgroup analyses performed. The mortality analyses presented below include 70 subgroups defined by baseline demographic characteristics, infection data, and disease severity measures. An estimated 5 of these 70 subgroups would be expected to have higher mortality in the effective treatment arm by chance alone even if drotrecogin alfa (activated) in truth were associated with a 20% relative risk reduction uniformly across all sub-populations of Study F1K-MC-EVAD. This estimate was derived from trial simulation using the observed placebo subgroup mortality rates, placebo and treatment subgroup sample sizes, and an assumed constant 0.80 relative risk for all subgroups with allowances for correlation among subgroups.
- The subgroup analyses presented contain no adjustments for potential baseline imbalances between treatment groups. Though randomization promotes baseline balance, randomization does not ensure baseline balance. In addition, the ability of randomization to promote baseline balance is reduced in smaller subgroups.

9.3. Benefit Analyses by Subgroup

A number of prospectively defined subgroup analyses were performed to assess the consistency of the drotrecogin alfa (activated) treatment effect on mortality across subgroups.

Analyses were performed for subgroups defined by baseline demographic characteristics, infection data, and disease severity measures. A within subgroup result was defined to be consistent with the overall trial result if the 95% confidence interval for the relative risk estimate within the subgroup contained the overall relative risk point estimate of 0.806 observed for the entire population. In addition, potential treatment-by-subgroup interactions were assessed using the Breslow-Day test for homogeneity of odds ratios across strata (Breslow and Day 1980).

9.3.1. Mortality by Demographic Characteristics

Mortality results across subgroups defined by patient demographic data, recent surgery status (within 30 days of study entry), and site and type of infection are presented in Figure 9.2. Consistently lower 28-day all-cause mortality for drotrecogin alfa (activated) patients compared with placebo patients was observed across subgroups defined by age, gender, racial origin, and recent surgery status (defined as surgery within the previous 30

days). Lower mortality rates were also observed with drotrecogin alfa (activated) administration within each geographic region. The largest number of patients were enrolled in the United States (N=705); treatment with drotrecogin alfa (activated) was associated with a 25.65% relative risk reduction in 28-day all-cause mortality compared to the placebo group (24.43% versus 32.86%, respectively).

9.3.2. Mortality by Site and Type of Infection

Investigators were asked to assess the presumed site of infection for patients enrolled into Study F1K-MC-EVAD. Lower mortality was observed for drotrecogin alfa (activated) patients with lung and intra-abdominal sites of infection (Figure 9.2). Compared to placebo patients with a presumed urinary tract infection as their primary infection site, slightly higher mortality was observed in drotrecogin alfa (activated) patients. As displayed by their relative risk confidence intervals, the subgroup results by the presumed site of infection were consistent with that observed in the overall trial. Lower mortality was observed for drotrecogin alfa (activated) patients compared with placebo patients regardless of the type of pathogen associated with the infection.

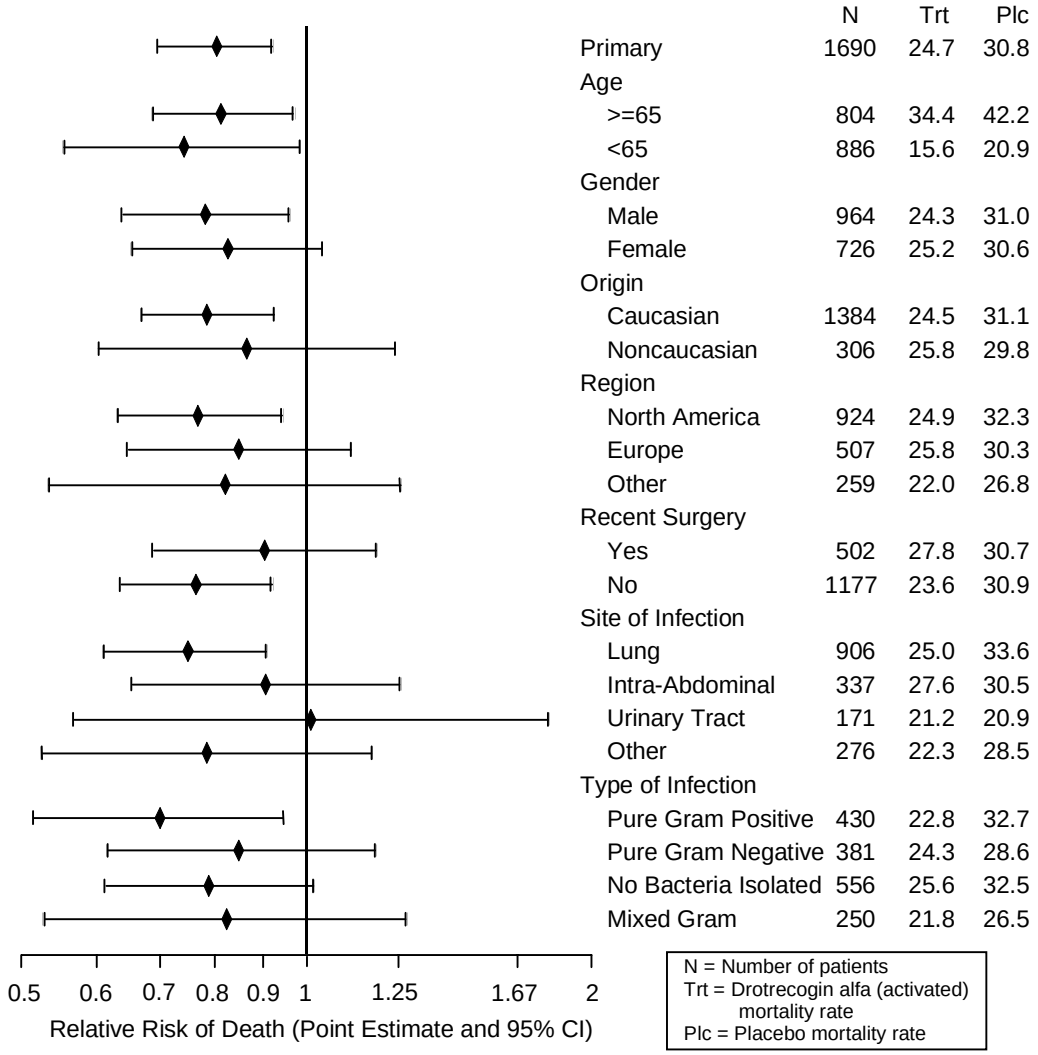


Figure 9.2. **28-day all-cause mortality across subgroups defined by patient baseline characteristics. Study F1K-MC-EVAD.**

9.3.3. Mortality by Baseline Disease Severity

Several analyses were performed with subgroups defined by clinical and biochemical markers of disease severity. Figure 9.3 presents the mortality results across subgroups

defined by a variety of clinical measures of baseline disease severity. The APACHE II score was calculated based on the most aberrant physiologic variable obtained within the 24-hour period immediately preceding study drug administration (Appendix 1). Cardiovascular, respiratory, hematologic, renal, and metabolic organ failures were those defined by the inclusion criteria (Appendix 5). The classification "Yes" indicated the presence of the organ failure within the 48-hour period immediately preceding administration of study drug. Number of organ failures was defined by the number of inclusion criteria organ failures present within this 48-hour period. Shock was defined as the presence of cardiovascular organ failure. A patient was classified as having "any shock" if the patient had any cardiovascular organ failure (as defined by the inclusion criteria) or a cardiovascular SOFA score >1. SOFA scores were based on the most aberrant physiologic variable obtained within 24 hours of study drug administration (Appendix 2).

A consistent treatment effect on 28-day all-cause mortality for drotrecogin alfa (activated) patients compared with placebo patients was observed based on these subgroups (Figure 9.3). Lower mortality rates were observed for drotrecogin alfa (activated) patients compared with placebo patients in all subgroups with the exception of the first APACHE II score quartile. The Breslow-Day treatment-by-APACHE-II-quartile interaction p-value was 0.09.

Figure 9.4 presents the mortality results across subgroups defined by a variety of biochemical measures of baseline disease severity. These biochemical measures were obtained within 24 hours prior to the start of study drug.

A consistent treatment effect on 28-day all-cause mortality for drotrecogin alfa (activated) patients compared with placebo patients was observed in all subgroups except for the first IL-6 quartile. For patients within the first IL-6 quartile, a subgroup of patients with relatively less inflammation at baseline than the overall population, the observed relative risk of death associated with drotrecogin alfa (activated) compared to placebo was 0.473. The relative risk 95% confidence interval for this subgroup (0.292 to 0.768) did not include the point estimate for the entire population (0.806). The Breslow-Day treatment-by-IL-6-quartile interaction p-value was 0.07. In all subgroups defined by biochemical measures of disease severity, lower mortality was observed for drotrecogin alfa (activated) patients compared with placebo patients.

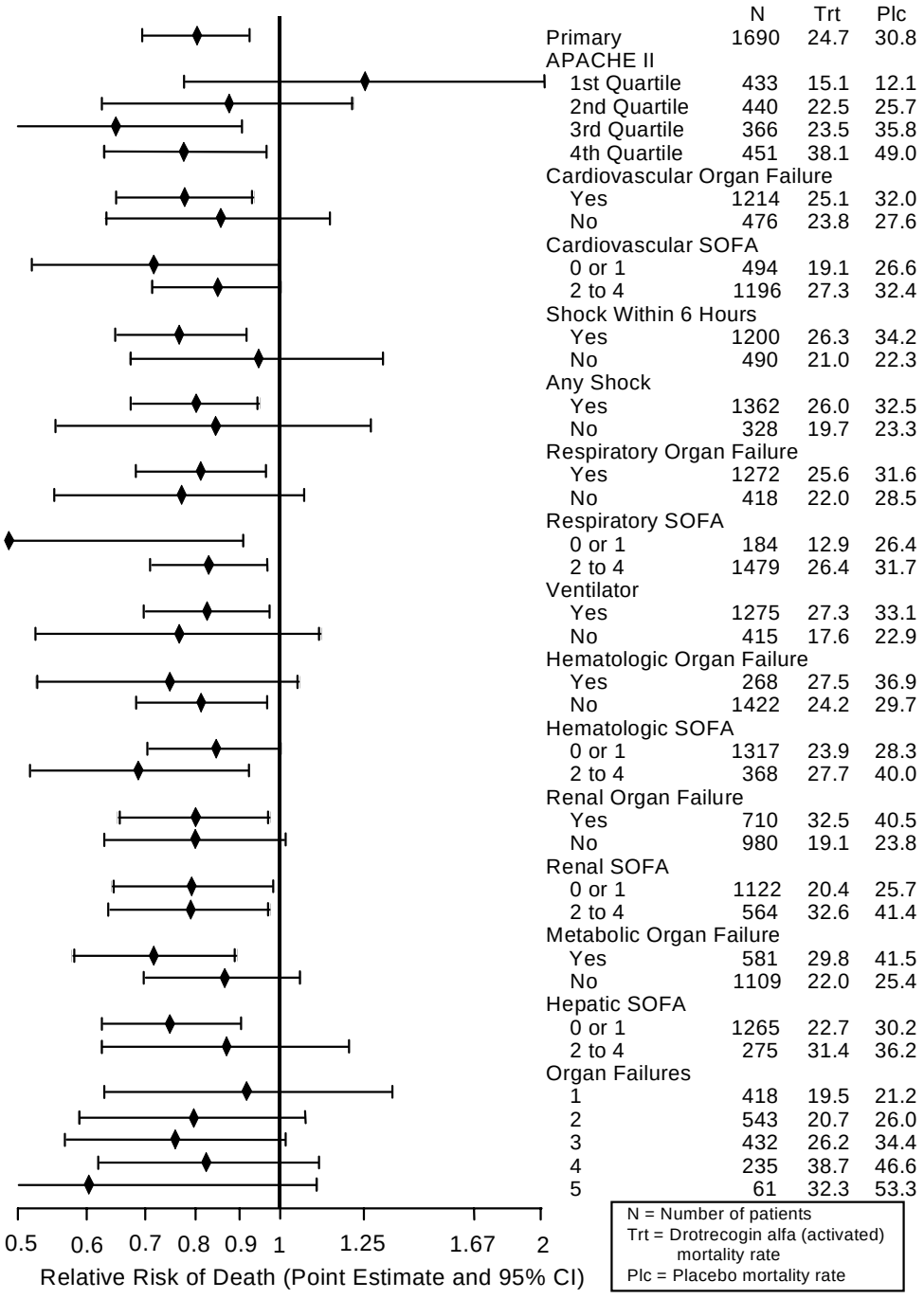


Figure 9.3. **28-day all-cause mortality across subgroups defined by clinical measures of baseline disease severity. Study F1K-MC-EVAD.**

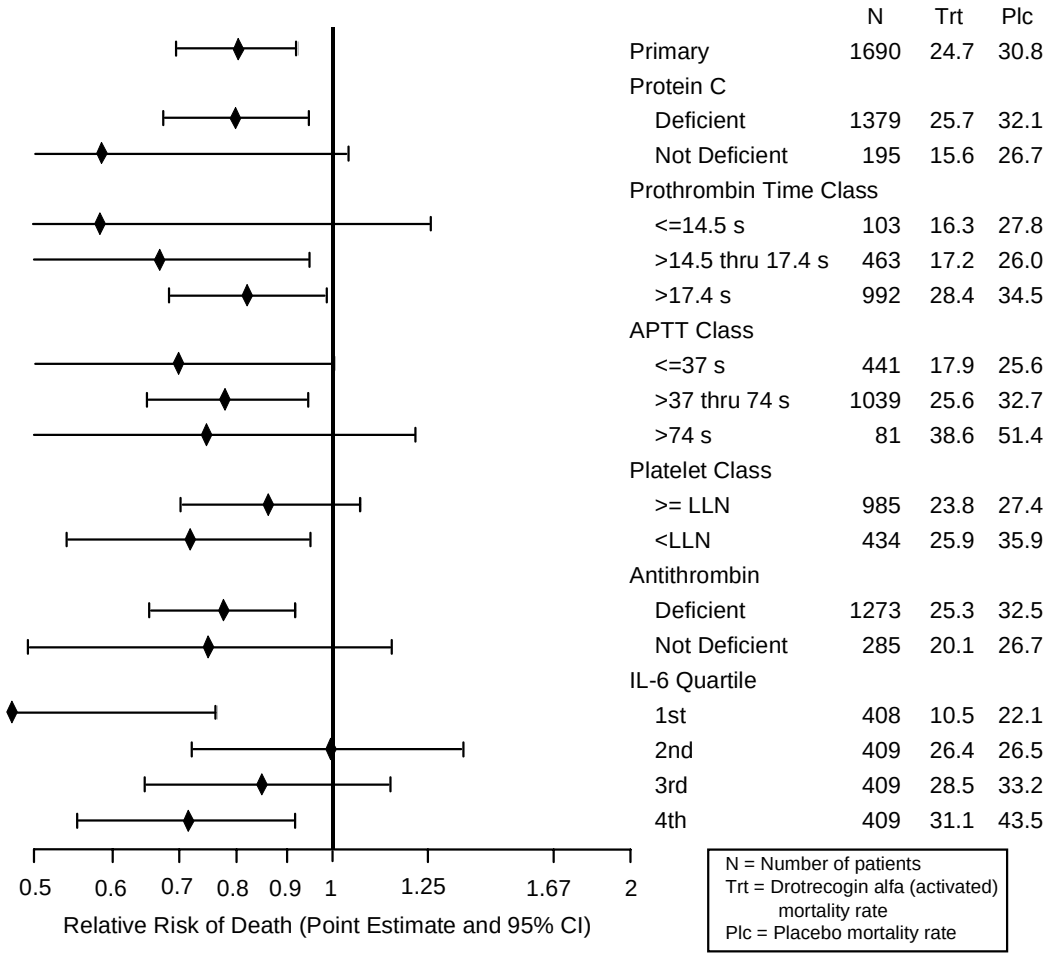


Figure 9.4. **28-day all-cause mortality across subgroups defined by biochemical measures of baseline disease severity. Study F1K-MC-EVAD.**

The observations in the first APACHE II quartile may lead to some conjecture that there is less treatment benefit in patients with less disease severity. However, the Study F1K-MC-EVAD results do not support such a hypothesis from either a statistical perspective or a clinical perspective:

- From a statistical perspective, the variation of the within-subgroup relative risk point estimates observed in Study F1K-MC-EVAD are remarkably consistent with what one would expect due to chance variation alone.

- From a clinical perspective, there is no pattern of a lesser treatment benefit across subgroups with less disease severity.
- From a clinical perspective, examination of results within the first APACHE II quartile subgroup do not support such a hypothesis.

As stated in the statistical caveats at the beginning of this section, if treatment were in truth associated with a constant 0.80 relative risk for all 70 subgroups, one would expect to observe higher mortality in the effective treatment group for five subgroups by random chance alone. In Study F1K-MC-EVAD, only two such subgroups were observed (urinary tract infection and first APACHE II quartile). Hence, from a statistical perspective, the number of extreme subgroup results in disfavor of drotrecogin alfa (activated) observed in Study F1K-MC-EVAD is less than would be expected by mere chance alone if the treatment were uniformly beneficial.

In theory, the relative risk point estimate will be less precise for subgroups with lower underlying placebo mortality rates compared to subgroups with higher underlying mortality rates and the same sample size. Thus, if the treatment benefit is constant across subgroups defined by disease severity, a tighter cluster in relative risk estimates around the overall risk estimate should be expected for subgroups with higher disease severity (ie, higher placebo mortality rates) than for subgroups defined by lower disease severity (ie, lower placebo mortality rates). Hence, more extreme relative risk point estimates in favor or disfavor of the beneficial treatment might be observed in lower disease severity subgroups (Figure 9.5). This phenomena is observed in Study F1K-MC-EVAD, with extreme results both in favor of and disfavor of drotrecogin alfa (activated) in subgroups with lower disease severity (Figure 9.5).

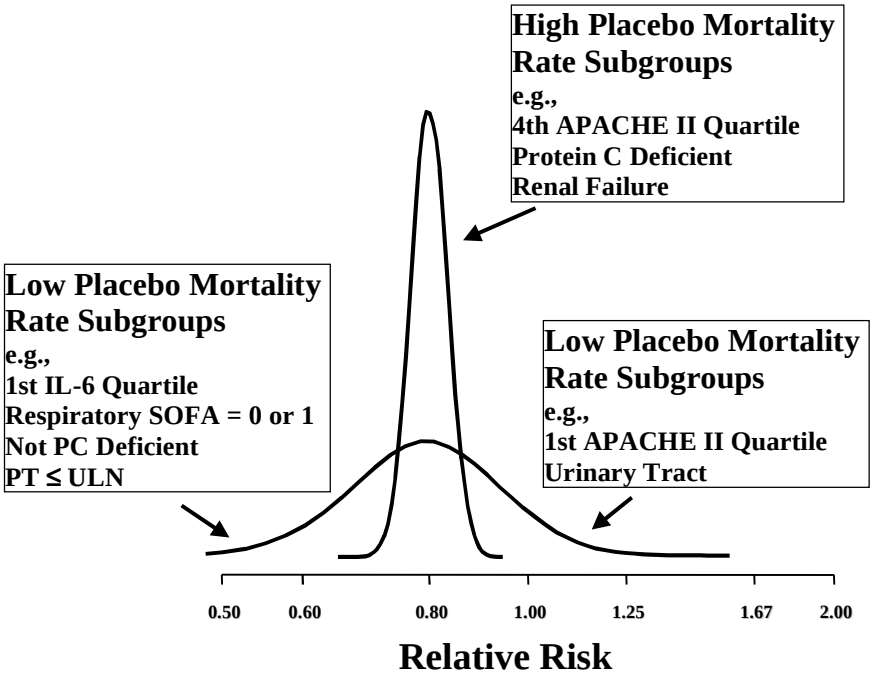


Figure 9.5. Illustration of theoretical variability in relative risk estimates by disease severity.

Figure 9.6 displays the relative risk point estimates and 95% confidence intervals for the subgroups with lower disease severity that were presented in Figures 9.3 and 9.4. Lower mortality was observed with drotrecogin alfa (activated) treatment for 20 of the 21 subgroups. In addition,12 of the 21 subgroups have larger observed relative risk reductions with treatment than was observed in the entire population. Hence, no predominant pattern of lesser treatment effect was observed across subpopulations of less disease severity.

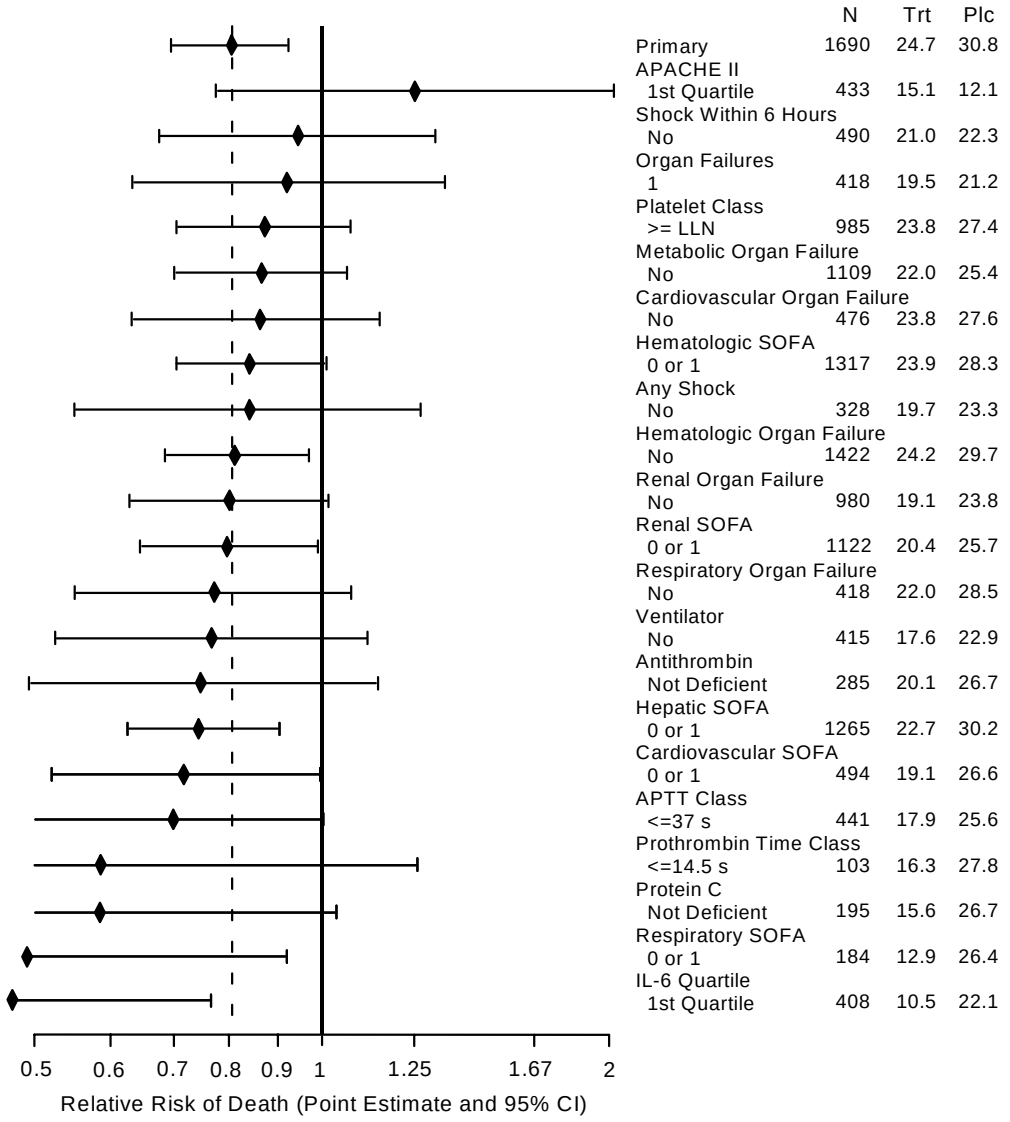


Figure 9.6. **28-day all-cause mortality across lower disease severity subgroups. Study F1K-MC-EVAD.**

Abbreviations: N = number of patients; Plc = placebo mortality rate; Trt = drotrecogin alfa (activated) mortality rate.

9.3.3.1. First APACHE II Quartile

For patients in the first APACHE II quartile, 60% had two or more organ failures at baseline, nearly 60% were receiving mechanical ventilation, and nearly 60% met the cardiovascular/shock entry criteria. Approximately 65% of patients were classified as severely Protein C deficient (Protein C activity <65%) at baseline. The apparent discrepancy between the relatively low APACHE II score and the high percentage of patients with shock and multiple organ failure may relate to the method by which the APACHE II score was obtained. The baseline APACHE II score was based on the most aberrant clinical and laboratory values obtained within the 24-hour period immediately preceding the administration of study drug. In contrast, the APACHE II scoring system was developed using the most aberrant values obtained within the first 24 hours of admission to the intensive care unit (ICU). Consequently, for patients enrolled in the study 2 or more days following ICU admission, the APACHE II score may not have encompassed the period of resuscitation. Therefore, many patients included within the first APACHE II score quartile for this trial were severely ill despite their relatively low APACHE II score.

To further assess whether there is evidence of a differential treatment effect with drotrecogin alfa (activated) by disease severity, an exploration of study results within the first APACHE II quartile was conducted (Table 9.1).

Table 9.1. Mortality Results for Subpopulations within the First APACHE II Quartile

Subgroup	N	Drotrecogin alfa (activated) Mortality	Placebo Mortality	Relative Risk (95% CI)
Number of Organ Failures				
≤ 1	167	6.67%	7.79%	0.86 (0.29 to 2.54)
≤ 2	322	9.7%	12.1%	0.80 (0.43 to 1.51)
≥ 3	111	32.1%	12.1%	2.66 (1.20 to 5.90)
IL-6 Levels[a]				
Below Median[b]	258	10.7%	13.4%	0.80 (0.41 to 1.55)
Above Median[b]	160	20.7%	11.5%	1.80 (0.85 to 3.79)

Abbreviations: CI = confidence interval; N = number of patients.

[a] Includes patients with available baseline measurements.

[b] The median of 492 pg/mL is based on the entire Study F1K-MC-EVAD patient population.

For the 167 patients in the first APACHE II quartile who had a single organ failure, a 14% relative risk reduction was observed with the administration of drotrecogin alfa

(activated). For the 322 patients in the first APACHE II quartile who had two or fewer organ failures, a 20% relative risk reduction was observed. The population of patients with two or fewer organ failures included approximately 75% of all patients in the first APACHE II quartile.

The subgroup of patients with three or more organ failures apparently drove the higher observed mortality for drotrecogin alfa (activated) within the first APACHE II quartile. The 32.1% observed mortality rate in drotrecogin alfa (activated) patients with three or more organ failures in the first APACHE II quartile is slightly higher than that observed for all drotrecogin alfa (activated) patients with three or more organ failures (30.8 %, N=364). In addition, the 12.1% mortality rate observed for placebo patients with three or more organ failures within the first APACHE II quartile is very divergent from that observed for all placebo patients with three or more organ failures (39.8 %, N=364).

A similar observation is made when the first APACHE II quartile subgroup is examined by IL-6 levels. For the 258 patients in the first APACHE II quartile who had IL-6 levels below the median of the overall study population, a 20% relative risk reduction was observed.

These data indicate that, for patients within the first APACHE II quartile with less severe disease, the administration of drotrecogin alfa (activated) was associated with a relative risk reduction similar to that observed in the overall population.

Taken together, these data provide no clear evidence of a differential treatment effect with drotrecogin alfa (activated) based on baseline disease severity, as assessed by the relative risk of death. Furthermore, the potential hypotheses that might underlie a differential effect by disease severity are not supported by the data discussed above. The finding of consistent and similar relative risk reductions associated with drotrecogin alfa (activated) in patients with low IL-6 levels (ie, less inflammation) and normal coagulation parameters (ie, prothrombin time, APTT, platelet count, Protein C activity, antithrombin activity) suggests that the absence of abnormalities in these parameters does not lessen the beneficial effect associated with drotrecogin alfa (activated) administration. Finally, the finding of consistent and similar relative risk reductions associated with drotrecogin alfa (activated) within multiple subgroups defined by a number of clinically relevant measures of disease severity also suggests no clear evidence of differential treatment effect by disease severity.

9.4. Risk Analyses by Subgroups

The potential for a differential treatment effect on the rate of bleeding events during the 28-day study period was investigated across the following subgroups: age, gender, racial origin, recent surgery status, baseline renal function, baseline hepatic function, APACHE II quartile, and number of organ failures. Similar analyses were performed for subgroups defined by coagulation parameters (APTT, PT, and platelets) at baseline and during the study drug infusion period. The same caveats regarding subgroup analyses for

mortality also apply to subgroup analyses investigating safety. Due to the low number of serious bleeding events observed in the study, the interaction tests presented have very low power to detect differential effects and can be unreliable.

Table 9.2 summarizes the percentage of patients (survivors and non-survivors) experiencing a serious bleeding event for the subgroups listed above by treatment group. There were no significant treatment-by-subgroup interactions observed for serious bleeding events for subgroups defined by age, gender, racial origin, recent surgery status, renal function, hepatic function, and number of organ failures. A treatment-by-APACHE II-quartile interaction (p=0.07) was present and resulted from a low percentage of drotrecogin alfa (activated) patients in the second APACHE II quartile who experienced a serious bleeding event. There was no treatment-by-APACHE II interaction for serious bleeding events when the APACHE II score was analyzed as a continuous covariate (p=0.72).

Table 9.2. Serious Bleeding Events by Subgroups Study F1K-MC-EVAD

	Drotrecogin Alfa (activated)		Placebo		
Subgroup	No. of Patients	Events n (%)	No. of Patients	Events n (%)	Interaction p-Value
Overall	850	30 (3.53)	840	17 (2.02)	
Gender					0.67
Female	373	18 (4.83)	353	11 (3.12)	
Male	477	12 (2.52)	487	6 (1.23)	
Age					0.86
<65	437	18 (4.12)	449	11 (2.45)	
≥65	413	12 (2.91)	391	6 (1.53)	
Origin					0.15
Caucasian	695	21 (3.02)	689	15 (2.18)	
Noncaucasian	155	9 (5.81)	151	2 (1.32)	
Recent Surgery					0.87
No	597	21 (3.52)	580	12 (2.07)	
Yes	245	9 (3.67)	257	5 (1.95)	
Renal OF					0.10
No	493	18 (3.65)	487	6 (1.23)	
Yes	357	12 (3.36)	353	11 (3.12)	
Hepatic SOFA					0.27
0 or 1	640	19 (2.97)	625	14 (2.24)	
2 to 4	137	9 (6.57)	138	3 (2.17)	
PT Class					0.76
0–14.5 s	49	0	54	0	
>14.5–17.4 s	232	6 (2.59)	231	4 (1.73)	
>17.4 s	514	22 (4.28)	478	11 (2.30)	
APTT Class					0.60
0–37 s	218	4 (1.83)	223	4 (1.79)	
>37–74 s	532	23 (4.32)	507	11 (2.17)	
>74 s	44	1 (2.27)	37	1 (2.70)	
Platelet Class					1.00
<LLN	228	9 (3.95)	206	4 (1.94)	
≥LLN	488	14 (2.87)	497	7 (1.41)	
Number of Organ Failures					0.92
0	1	0 (0)	0	0 (NA)	
1	215	6 (2.79)	203	2 (0.99)	
2	270	6 (2.22)	273	4 (1.47)	
3	214	12 (5.61)	218	7 (3.21)	
4	119	6 (5.04)	116	4 (3.45)	
5	31	0 (0)	30	0 (0)	
APACHE II Quartile					0.07 Logistic p=0.72
1st	218	10 (4.59)	215	2 (0.93)	
2nd	218	4 (1.83)	222	8 (3.60)	
3rd	204	9 (4.41)	162	3 (1.85)	
4th	210	7 (3.33)	241	4 (1.66)	

To further assess the risk of serious bleeding in patients with a developing or worsening coagulopathy during the study drug infusion, the most extreme measures of coagulopathy observed during study drug infusion were assessed. An analysis of serious bleeding events by treatment group and maximum observed APTT during the study drug infusion was performed. Since the maximum observed APTT during infusion is a postbaseline result potentially effected by the treatment received, caution must be used in drawing conclusions from this analysis. Indeed, a consequence of the effect of drotrecogin alfa (activated) on APTT levels is a higher percentage of drotrecogin alfa (activated) patients classified into the highest maximum APTT stratum compared with placebo patients. Similar analyses were performed for maximum PT and minimum platelet count observed during the study drug infusion period.

The percentage of patients who experienced a serious bleeding event increased during infusion in both the drotrecogin alfa (activated) and the placebo groups with prolonged APTT or prolonged PT. However, the odds of experiencing a serious bleeding event during the 28-day study period did not increase with increasing coagulopathy as defined by maximum APTT, PT or minimum platelet count observed during the study drug infusion.

Because of the low incidence of serious bleeding events, additional risk assessments based on all serious and non-serious bleeding events were conducted. The incidence of treatment-emergent bleeding events was examined across all 70 subgroups defined for the mortality analyses to further assess potential differential treatment effects on the odds of experiencing a bleeding event. Based on Breslow-Day tests, there were no clinically relevant treatment-by-subgroup interactions observed for treatment-emergent bleeding events across any of the subgroups assessed.

To summarize risk, drotrecogin alfa (activated) compared to placebo increased the risk of serious and non-serious bleeding events. However, there was no evidence of a differential treatment effect on the relative risk or odds of bleeding across the subgroups assessed.

9.4.1. Conceptual Benefit-Risk Assessment by APTT

Table 9.3 conceptualizes, from a simplified perspective, the benefit risk assessments a physician may encounter with respect to two patients with different APTT levels. The patient with an elevated APTT has a higher underlying bleeding risk than the patent with a low APTT; therefore, from a population perspective, the excess bleeding risk associated with drotrecogin alfa (activated) treatment would be greater for the prolonged APTT patient. However, it is also true that the elevated APTT patient has a higher underlying probability of death than the low APTT patient; thus, from a population perspective, the anticipated survival benefit of drotrecogin alfa (activated) treatment also would be greater for the prolonged APTT patient. Importantly, based on any risk benefit assessment, the

net benefit associated with drotrecogin alfa (activated) treatment would be positive for both patients.

Table 9.3 Conceptual Benefit-Risk Assessment by APTT

	Low APTT Patient (<37 secs)	High APTT Patient (>74 secs)	Comment
Underlying Serious Bleeding Risk	1.8%	2.7%	Based on observed F1K-MC-EVAD placebo serious bleeding rates
Underlying Mortality Risk	25.0%	50.0%	Based on observed F1K-MC-EVAD placebo mortality rates
Absolute Excess Bleeding Risk	1.35%	2.03%	Assumed constant 1.75 RR based on observed overall F1K-MC-EVAD RR
Absolute Survival Benefit	5%	10%	Assumed constant 0.80 RR based on observed overall F1K-MC-EVAD RR
Net Benefit	Positive	Positive	

Abbreviations: RR = Relative Risk.

9.5. Summary

There was a consistent effect of drotrecogin alfa (activated) on the relative risk of death across subgroups defined by baseline patient demographics, infection site, infection type, and clinical and biochemical measures of disease severity. The administration of drotrecogin alfa (activated) was associated with an increased incidence of serious bleeding events. However, the small absolute increase in the number of patients experiencing a serious bleeding event (n=13) precluded a robust assessment of bleeding risk by subgroups.

Figure 9.7 summarizes the net benefit-risk associated with drotrecogin alfa (activated) treatment by true underlying placebo mortality rates. The abscissa range of 10% to 50% placebo mortality encompasses the range of placebo mortality rates observed in the 70 subgroups that were assessed.

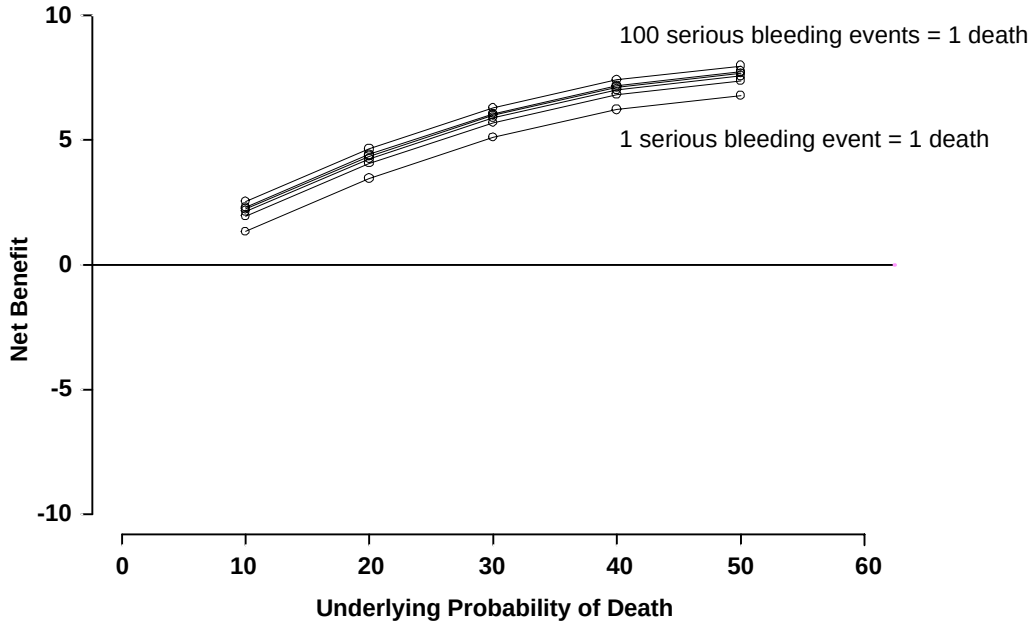


Figure 9.7. **Net benefit-risk of treatment by underlying (unknown) probability of death. Study F1K-MC-EVAD.**

In this analysis, benefit equals 38.1% increased odds of survival and risk equals 1.2% absolute increase in serious bleeding events for survivors.

Regarding benefit, there is no clear evidence of a differential treatment effect on the relative risk or odds of death based on demographics, infection information, and clinical and biochemical markers of disease severity. Thus, for Figure 9.7, a constant 38.1% increase in the odds of survival with drotrecogin alfa (activated) was applied across the range of underlying mortality rates.

Regarding risk, there is no clear evidence of a differential treatment effect on the odds of experiencing a bleeding event based on the same 70 subgroups. Furthermore, no association between the underlying risk of a bleeding event and the underlying risk of death in the placebo group is apparent. Thus, for Figure 9.7, a constant 1.2% increase in the absolute rate of serious bleeding events in survivors was applied across the range of underlying mortality rates.

In summary, assessments of net benefit-risk with drotrecogin alfa (activated) treatment by underlying probability of death rates is displayed in Figure 9.7. Regardless of how one weighs the risk of an additional serious bleeding event in survivors to the benefit of saving one additional life, drotrecogin alfa (activated) treatment is associated with a

positive benefit risk profile across the range of underlying probability of deaths one would expect for the differing populations of patients enrolled within Study F1K-MC-EVAD. That is, the life saving potential of drotrecogin alfa (activated) outweighs the risks associated with its use.

10. Immunogenicity

The amino acid sequence of drotrecogin alfa (activated) and plasma-derived Activated Protein C are identical. The carbohydrate portion of drotrecogin alfa (activated) contains unique oligosaccharides that are not detectable in plasma-derived Activated Protein C. These unique oligosaccharides have been detected in several naturally occurring human glycoproteins and are unlikely to be antigenic (Yan et al. 1993). Nevertheless, immune response in subjects and patients exposed to drotrecogin alfa (activated) was monitored.

Immune response in patients was determined using a three-tiered testing methodology:

- Level 1 Assay: Detection of non-specific anti-Activated Protein C antibody response. A sequential solid-phase chemiluminescent binding assay was used to screen serum samples for anti-Activated Protein C immunoglobulin G (IgG) antibodies. The serum sample was diluted 1:10 for this assay. A positive control of rabbit anti-human Protein C antibody was included in each assay run. The results are reported in Adjusted Relative Light Units (ARLU). A significant increase or positive response was defined as a 2-fold or greater rise from baseline value (predose sample) coupled with an ARLU value for the sample of >124 (the lower limit of quantitation). Samples with a positive result progressed to Level 2 testing.
- Level 2 Assay: Detection of specific anti-Activated Protein C antibody response. This inhibition assay uses the sequential solid-phase binding methodology, adding exogenous drotrecogin alfa (activated) to compete for binding to any anti-Activated Protein C antibodies present in serum. This assay is quantitated in ARLU. A sample with greater than 40% inhibition is considered to contain specific anti-Activated Protein C IgG antibodies. Positive Level 2 samples were further evaluated by a final Level 3 testing.
- Level 3 Assay: Detection of neutralizing anti-Activated Protein C antibodies was performed using a two-step neutralizing antibody assay. IgG from the patient plasma sample is isolated and used in an APTT activity measurement of drotrecogin alfa (activated) and plasma human Activated Protein C. A neutralizing antibody would be expected to diminish the effect of Activated Protein C on the APTT.

Anti-Activated Protein C antibody response data were monitored in 105 unique subjects who participated in the eight Phase 1 studies. The anti-Activated Protein C antibody data were available from 104 of the 105 subjects exposed to drotrecogin alfa (activated) (samples from Study F1K-LC-GUAF Subject 1336 were lost). A majority (90/104, 87%) of the subjects in which immune response was monitored were exposed to drotrecogin alfa (activated) more than once. A total of 19 subjects out of 104 subjects (18%) received drotrecogin alfa (activated) four to six times. The time interval between re-exposure ranged from less than 7 days (7%) to more than 90 days (20%). No anti-Activated

Protein C antibody response was detected in any of the Phase 1 subjects dosed with drotrecogin alfa (activated), even with multiple re-administration.

In the Phase 2/3 studies, the overall incidence of anti-Activated Protein C antibody response in patients exposed to drotrecogin alfa (activated) was 0.54% (2/370 patients). The anti-Activated Protein C antibodies that developed in these 2 patients were non-neutralizing.

In Study F1K-MC-EVAA, patients were monitored for an immune response at 14 days following the start of study drug infusion. Of the 90 drotrecogin alfa (activated) patients , 53 had samples for anti-Activated Protein C antibody testing. Two of these patients, Patients 003-0304 (24 μg/kg/hr for 48 hours) and Patient 015-1501 (12 μg/kg/hr for 48 hours) had positive Level 1 results. Both patient samples were tested for specific anti-Activated Protein C antibodies (Level 2 testing). Only Patient 003-0304 had a positive Level 2 response. Level 3 testing for this patient was negative. The immune response of Patient 003-0304 was transient. A sample taken about a year after the initial drotrecogin alfa (activated) exposure was found to be negative for anti-Activated Protein C antibody response. The development of drotrecogin alfa (activated) antibody response in Patient 003-0304 was not associated with any clinical adverse events.

In Study F1K-MC-EVAD, 310 patients [including the 2 patients in the placebo treatment group who received drotrecogin alfa (activated) for some length of time] had samples for Level 1 anti-Activated Protein C antibody testing collected at baseline and between Study Days 12 and 21. A total of 237 patients [including 1 of the 2 patients in the placebo treatment group who received drotrecogin alfa (activated) for some length of time] had samples for Level 1 anti-Activated Protein C antibody testing collected at baseline and on or after Study Day 22. In aggregate, 317 patients had a sample for Level 1 anti-Activated Protein C antibody testing collected at baseline and at least one sample collected on or after Study Day 12.

Three patients had positive Level 1 results (Patients 045-4502, 340-4003, and 851-5110). However, only 1 patient (Patient 340-4003) had specific anti-Activated Protein C antibodies (positive Level 2 result) detected on the Study Day 28 sample. This patient did not develop Activated Protein C neutralizing antibodies (negative Level 3 result) but did experience superficial and deep venous thromboses that were not deemed serious by the investigator. This patient was alive at Study Day 28.

11. Experience in Pediatric Patients

11.1. Regulatory History

Lilly has worked closely with the United States Food and Drug Administration ("the Agency") to design and implement a clinical development plan that evaluates the safety and effectiveness of drotrecogin alfa (activated) in pediatric patients with severe sepsis, as detailed below.

(1) A proposed pediatric study and targeted labeling were submitted for review to the Agency in October 1999. In accordance with the pediatric rule, the objectives of the pediatric study were:

- To provide evidence (in addition to the available published literature) that the course of pediatric sepsis is similar to severe sepsis in adult patients. Comparability of sepsis between the two patient populations would be assessed by clinical evidence, clinical history, and determination of baseline coagulation markers, including D-dimer, Protein C, and antithrombin.
- To demonstrate that the beneficial effect of drotrecogin alfa (activated) is similar in pediatric patients compared to adult patients. Comparability of effect would be assessed by measuring changes in plasma D-dimer concentrations.
- To ascertain that the safety profile of drotrecogin alfa (activated) (that is, bleeding complications) is similar in pediatric patients compared to adult patients.
- To provide adequate pharmacokinetic data to support dosing and administration of drotrecogin alfa (activated) in pediatric patients with sepsis, age newborn to <18 years.
- To obtain additional data regarding safety of drotrecogin alfa (activated) in pediatric patients at the recommended dose and duration.

(2) Lilly proposed that if the study's objectives were met, effectiveness of treatment with drotrecogin alfa (activated) in the pediatric population could be extrapolated from results of the Phase 3 study in adults. Accordingly, the proposed pediatric study would support the indication of drotrecogin alfa (activated) in pediatric patients with sepsis.

(3) A teleconference was held with the Agency in November 1999 to discuss the proposed pediatric study and Lilly's plan for obtaining data in pediatric patients to support a pediatric indication. An agreement was reached to evaluate children categorized according to three age groups (newborn to <1 year, 1 to <8 years, and 8 to <18 years). Furthermore, there was concurrence that, should the Phase 3 study in adults show a treatment benefit of drotrecogin alfa (activated) upon mortality, it may not be possible to conduct a placebo-controlled efficacy study in pediatric patients with sepsis for ethical reasons. Hence, extrapolation of the efficacy data from the adult population to pediatrics would be feasible, if the Phase 3 study were successful, and if adequate pharmacokinetic and safety data were obtained in pediatric patients.

(4) The protocol for the pediatric study, entitled "Investigation of the Safety, Pharmacokinetics and Pharmacodynamics of Drotrecogin Alfa (Activated) in Pediatric Sepsis" (Study F1K-MC-EVAO) was filed with the Agency in December 1999.

(5) Because of the early termination of the adult Phase 3 study in July 2000, the Biologics License Application (BLA) for use of drotrecogin alfa (activated) in adult patients with severe sepsis was submitted to the Agency prior to completion of the pediatric study.

(6) The final clinical study report for the pediatric study will be forthcoming and filed with the Agency as soon as possible after the BLA action date.

11.2. Experience in Pediatric Patients

This section contains a summary of a review of the literature on pediatric severe sepsis and a summary of data from ongoing trials being conducted by Lilly in pediatric patients with severe sepsis, adult and pediatric patients with purpura fulminans, and adult and pediatric patients with severe sepsis. This data comes primarily from Study F1K-MC-EVAO, which is an open-label, safety, pharmacokinetic, and pharmacodynamic study of drotrecogin alfa (activated) in pediatric patients with severe sepsis (age newborn to <18 years). Additional safety data from Study F1K-MC-EVAS, a compassionate use trial in adult and pediatric patients with purpura fulminans; Study F1K-MC-EVBC, a treatment use trial in adult and pediatric patients with severe sepsis, and Studies F1K-MC-EVBE, F1K-MC-EVBF, and F1K-MC-EVBG, which are open-label studies in adult and pediatric patients with severe sepsis (Section 11.8). All of these studies are currently ongoing.

11.3. Literature Review

A review of the literature demonstrates that the pathophysiology and disease state of sepsis appears to be similar between adults and children (see Appendix 4 for references of the articles reviewed). In summary, both adults and children display symptoms that indicate a systemic inflammatory response leading to coagulopathy, hypotension,

inadequate perfusion of peripheral tissues and organs, and ultimately organ failure and death. Adults and children do appear to differ in the absolute amount of some blood factors. However, there is no indication that the response to sepsis is qualitatively different in adults and children. Currently, no substantial differences are noted in the diagnosis or treatment of shock with the exception that the criteria for diagnosis in children are adjusted relative to inherent differences in vital signs such as heart rate, blood pressure, and respiratory rate. Essentially, the diagnostic guidelines established by the Society of Critical Care Medicine (SCCM) for adult sepsis are applied in an age sensitive manner by practicing pediatricians. The similarity in the disease state between adults and children is apparent in the nearly identical criteria used by researchers to identify sepsis in adults and children, and the similarity in diagnosis and treatment.

11.4. Pediatric Study F1K-MC-EVAO

11.4.1. Overview

Study F1K-MC-EVAO was an open-label, dose-escalation, dose-duration study in pediatric patients with severe sepsis. The objectives of the study were to investigate the safety, pharmacokinetics, and pharmacodynamics of drotrecogin alfa (activated) administration to pediatric patients with severe sepsis. The pharmacodynamics of drotrecogin alfa (activated) were assessed by changes in D-dimer concentration, Protein C activity level, and antithrombin activity level associated with the administration of drotrecogin alfa (activated).

Eligible patients were newborn (at least 38 weeks from estimated date of conception) to 17 years of age, inclusive, and met the following severe sepsis criteria: had a suspected or proven infection, had evidence of systemic inflammation, and had one or more sepsis-induced organ failures. The inclusion criteria in the pediatric study were fashioned after those used in the pivotal adult study (Study F1K-MC-EVAD) and are similar to those used in other pediatric trials of severe sepsis. The systemic inflammation and organ failure inclusion criteria for the pediatric patients were age specific and were recommended by the pediatric intensivists who participated in the development of the protocol. The definition of cardiovascular organ failure for the pediatric population was an arterial systolic blood pressure of <10th percentile for age after administration of approximately ≥ 20 mL/kg crystalloid or need for vasoactive support. Respiratory organ failure was defined in the original protocol as evidence of acute pulmonary dysfunction defined as a PaO_2/FiO_2 ratio of ≤200 and (if measured) a pulmonary capillary wedge pressure not suggestive of central volume overload.

The exclusion criteria were very similar to those used in the adult study, but were modified to exclude pediatric patients who may have potentially done well on standard therapy alone (ie, criteria defining organ failure were more severe for pediatric patients compared to adult patients.) These inclusion criteria were instituted to address the pediatric ethical imperative that every child enrolled in the study should have the

potential to benefit. In fact, the respiratory organ failure criterion for the pediatric patients was so strict that very few patients were enrolled with respiratory organ failure alone.

The patient population was segmented into three age groups: newborn to <1 year, 1 year to <8 years, and 8 years to <18 years. The study was conducted in two sequential parts within each age group:

- Part 1 was an open-label, dose-escalation, safety, and pharmacokinetic study in which drotrecogin alfa (activated) was administered as a 6-hour constant-rate infusion once daily on 4 consecutive days at infusion rates of 6, 12, 24, and 36 μg/kg/hr. Pharmacokinetic data were obtained for each infusion rate administered and were used to calculate an age-group specific infusion rate that was predicted to produce a steady-state Activated Protein C concentration of approximately 50 ng/mL. This age-group specific infusion rate was used in Part 2.
- Part 2 was an open-label, safety, pharmacokinetic, and pharmacodynamic study in which drotrecogin alfa (activated) was administered as a 24 μg/kg/hr constant-rate infusion for 96-hours. The infusion rate studied in Part 2 was based on an analysis of pharmacokinetic data obtained in Part 1.

The final drotrecogin alfa (activated) infusion rate recommendation for pediatric patients with severe sepsis will be based on the analysis of data from the entire study population (Parts 1 and 2 combined.)

11.4.2. Patient Disposition

Twenty-one patients were enrolled and received drotrecogin alfa (activated) in Part 1: 6 patients in the newborn to <1 age group, 8 patients in the 1 to <8 age group, and 7 patients in the 8 to <18 age group.

Data analysis and validation for Part 2 is currently ongoing. As of 31 May 2001, 62 patients had been enrolled and received drotrecogin alfa (activated): 19 patients in the newborn to <1 age group, 26 patients in the 1 to <8 age group, and 17 patients in the 8 to <18 age group.

The Phase 3 study in adult patients with severe sepsis (Study F1K-MC-EVAD) was completed after the pediatric study had begun. Following successful completion of Study F1K-MC-EVAD, enrollment criteria for Study F1K-MC-EVAO were modified by broadening the inclusion criteria to mirror more closely those used for the adult patients. The protocol was amended by adding two new organ failure criteria (renal and hematological) to be considered when assessing patients for eligibility. The respiratory organ failure criterion was changed to require a PaO_2/FiO_2 ratio of ≤250. The protocol amendment was approved on 5 October 2000. All patients in Part 1 were enrolled under

the original protocol; 30 patients in Part 2 were enrolled under the original protocol and 32 patients under the amended protocol.

11.4.3. Patient Characteristics

Table 11.1 contains a summary of baseline demographic and illness characteristics of pediatric patients enrolled in Part 1 and Part 2 of the study.

Table 11.1. Baseline Characteristics of Pediatric Patients Study F1K-MC-EVAO

Characteristic	Part 1 N=21	Part 2 N=62
Gender, n (%)		
Female	11 (52.4)	30 (48.4)
Male	10 (47.6)	32 (51.6)
Mean Age (yr)		
0 to <1 Year Age Group	0.32	0.36
1 to <8 Years Age Group	3.76	3.67
8 to <18 Years Age Group	12.92	14.32
Origin, n (%)		
African Descent	2 (9.5)	8 (12.9)
Caucasian	15 (71.4)	42 (67.7)
Hispanic	3 (14.3)	5 (8.1)
Other	1 (4.8)	7 (11.3)
Site of Infection, n (%)		
Blood	9 (42.9)	20 (32.3)
Cardiac	0	1 (1.6)
Central Nervous System	3 (14.3)	11 (17.7)
Gynecologic	0	3 (4.8)
Head/EENT	1 (4.8)	3 (4.8)
Intra-Abdominal	3 (14.3)	3 (4.8)
Lung	2 (9.5)	15 (24.2)
Urinary Tract	1 (4.8)	2 (3.2)
Vascular Catheter	0	2 (3.2)
Other	2 (9.6)	2 (3.2)
Type of Pathogen		
Gram Positive	5 (23.8)	11 (17.7)
Gram Negative	4 (19.0)	25 (40.3)
Mixed Gram	3 (14.3)	8 (12.9)
Organ Failures, n (%)		
Cardiovascular	19 (90.5)	56 (90.3)
Respiratory	5 (23.8)	24 (38.7)
Hematologic[a]	NA	8 (12.9)
Renal[a]	NA	4 (6.5)
Number of Organ Failures, n (%)		
1	18 (85.7)	39 (62.9)
2	3 (14.3)	18 (29.0)
3[a]	NA	3 (4.8)
4[a]	NA	2 (3.2)

Abbreviation: EENT = eye, ear, nose, throat; NA = not applicable.

[a] Under the original protocol, only cardiovascular and respiratory organ failures were recorded as baseline characteristics. Under the amended protocol, hematologic and renal organ failure were added to the inclusion criteria and were collected as baseline characteristics. Thus, the percentages given likely underestimate the hematologic and renal organ failure present in this population.

Source: PC10125#, PC10126#, PC10525#, PC10526#, PC10325#, PC10326#, PC10426#

Table 11.2 contains a comparison of the baseline illness characteristics of pediatric and adult patients with severe sepsis. The pediatric patient population is similar to the adult patient population in type of pathogen and type of organ failure present at baseline. However, there appeared to be a large number of pediatric patients with central nervous system listed as the site of infection, which is consistent with the higher incidence of meningitis in this population. There were fewer respiratory organ failures in the pediatric population, which is likely related to the strict respiratory organ failure inclusion criteria that was instituted in the original protocol.

Table 11.2. Comparison of Baseline Illness Characteristics of Pediatric and Adult Patients with Severe Sepsis Studies F1K-MC-EVAO and F1K-MC-EVAD

Characteristic	Pediatric Patients Parts 1 and 2 Combined Study F1K-MC-EVAO N=83		Adult Patients Study F1K-MC-EVAD N=1690	
Site of Infection, n (%)				
Blood	29	(34.9)	87	(5.1)
Cardiac	1	(1.2)	9	(0.5)
Central Nervous System	14	(16.9)	39	(2.3)
Gynecologic	3	(3.6)	8	(0.5)
Head/EENT	4	(4.8)	8	(0.5)
Intra-Abdominal	6	(7.2)	337	(19.9)
Lung	17	(20.5)	906	(53.6)
Urinary Tract	3	(3.6)	171	(10.1)
Catheter	2	(2.4)	15	(0.9)
Other	4	(4.8)	110	(6.6)
Type of Pathogen				
Gram Positive	16	(19.3)	430	(25.4)
Gram Negative	29	(34.9)	381	(22.5)
Mixed Gram	11	(13.3)	250	(14.8)
Organ Failures[a], n (%)				
Cardiovascular	29	(90.6)	1214	(71.8)
Respiratory	12	(37.5)	1272	(75.3)
Hematologic	8	(25.0)	268	(15.9)
Renal	4	(12.5)	710	(42.0)
Number of Organ Failures[a], n (%)				
1	18	(56.3)	418	(24.7)
2	9	(28.1)	543	(32.1)
3	3	(9.4)	432	(25.6)
4	2	(6.3)	235	(13.9)
5	NA		61	(3.6)

Abbreviations: N = number of patients; NA = not applicable.

[a] Pediatric patients (N=32) included in the comparison of organ failures were only those enrolled under the amended F1K-MC-EVAO(a) protocol to facilitate comparison with Study F1K-MC-EVAD.

Source: PC10326#, PC10426#, PC10525#, PC10526#, Table EVAD.11.3, Table EVAD.11.7.

Table 11.3 contains a summary of baseline biomarker data for Part 2 pediatric patients and adult patients with severe sepsis. The baseline biomarker data that describe the sepsis-induced coagulopathy for Part 2 pediatric patients in Study F1K-MC-EVAO are similar to data for adult patients enrolled in Study F1K-MC-EVAD.

Table 11.3. Baseline Biomarker Data for Pediatric and Adult Patients with Severe Sepsis Studies F1K-MC-EVAO and F1K-MC-EVAD

	Pediatric Study F1K-MC-EVAO Part 2 Patients			Adult Study FIK-MC-EVAD
Biomarker	0 to <1 years	1 to <8 years	8 to <18 years	≥18 years
Protein C Activity				
Number of Patients	16	25	16	1574
Median	0.23	0.37	0.28	0.48
Normal Range	>0.21	>0.51	>0.81	0.81 – 1.73
Antithrombin Activity				
Number of Patients	16	25	15	1558
Median	0.64	0.63	0.59	0.59
Normal Range	0.50 – 1.10	0.80 – 1.20	0.80 – 1.20	0.80 –1.20
D-Dimer Level (μg/mL)				
Number of Patients	16	25	15	1550
Median	2.91	7.23	6.19	4.21
Normal Range	0 – 0.39	0 – 0.39	0 – 0.39	0 – 0.39

Source: CM10126#, CM10226#, CM10326#.

Table 11.4 contains a comparison of the percentage of pediatric and adult patients with abnormal baseline levels of these biomarkers. Greater than 80% of pediatric and adult patients were Protein C deficient, greater than 65% of pediatric and adult patients were deficient in antithrombin, and nearly 100% of pediatric and adult patients had elevated (abnormal) D-dimer levels at baseline.

Table 11.4. Percentage of Patients with Baseline Biomarker Values Outside the Normal Range Studies F1K-MC-EVAO and F1K-MC-EVAD

Biomarker	Pediatric Patients Parts 1 and 2 Combined Study F1K-MC-EVAO	Adult Patients Study F1K-MC-EVAD
Protein C Activity		
Number of Patients	79	1574
Percent with values below the lower limit of normal	81.0	87.6
Antithrombin Activity		
Number of Patients	78	1558
Percent with values below the lower limit of normal	67.9	81.7
D-Dimer Level		
Number of Patients	78	1550
Percent with values above the upper limit of normal	100.0	99.7

Source: LB10325#, LB10326#.

Comparisons of baseline illness characteristics and biomarker data provide evidence that severe sepsis syndrome is similar in the pediatric and adult patients enrolled in the two trials.

11.4.4. Exposure

Table 11.5 contains a summary of the mean hours of exposure to drotrecogin alfa (activated) by age group in Part 2 of the study.

Table 11.5. Patient Exposure to Drotrecogin Alfa (activated) Part 2 Study F1K-MC-EVAO

Age group	Number of Patients	Patients with ≥72 Hours of Infusion	Mean Hours of Exposure to Drotrecogin Alfa (Activated)
0 to <1 years	19	14	75.3
1 to <8 years	26	22	85.1
8 to <18 years	17	8	61.4

Source: SD10726#

11.5. Pharmacokinetics

11.5.1. Part 1 Preliminary Results

Table 11.6 contains a summary of preliminary pharmacokinetic data from pediatric patients who received escalating infusion rates of drotrecogin alfa (activated) from 6 μg/kg/hr to 36 μg/kg/hr.

Table 11.6. Mean (CV%) Body Weight-Normalized Activated Protein C Clearance (L/hr/kg) in Pediatric Patients with Severe Sepsis Part 1 Study F1K-MC-EVAO

	Infusion Rate (μg/kg/hr)			
Age	6[a]	12	24	36
0 years to <1 year N=6	0.30 (43) n=3	0.66 (20) n=4	0.73 (16) n=6	0.62 (14) n=3
≥1 year to <8 years N=7	0.42 (39) n=7	0.58 (45) n=7	0.72 (45) n=7	0.64 (34) n=6
≥8 years to <18 years N=7	0.47 (17) n=6	0.57 (25) n=7	0.52 (17) n=5	0.56 (14) n=5

Abbreviations: Cp = plasma clearance; CV = coefficient of variation; N = number of patients per age group; n = number of patients per dose group.

a Based on 10 to 14 samples per age group, of which 3 to 5 per age group were below the limit of quantitation.

Plasma clearance (Cl_p) in all pediatric patients regardless of age or infusion rate was approximately 0.57 L/hr/kg. Body weight and age are confounded in the pediatric population, but the relationship between the body weight-normalized Cl_p and age suggests that age itself does not affect the Cl_p of Activated Protein C (Figure 11.1).

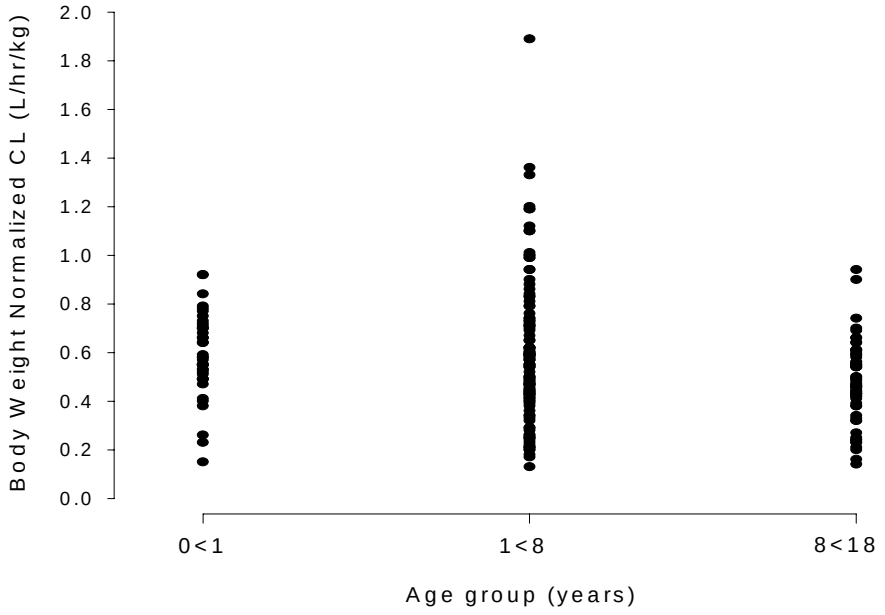


Figure 11.1. **Relationship between body weight-normalized Activated Protein C clearance and age across all three age groups for Part 1 of Study F1K-MC-EVAO.**

Based on a linear mixed-effect model, there was a strong relationship between Cl_p and body weight ($p<0.001$). Cl_p normalized to body weight was unchanged across the infusion rate range of 12 μg/kg/hr to 36 μg/kg/hr ($p>0.1$), but not across the range of 6 μg/kg/hr to 36 μg/kg/hr. However, mean estimates of Cl_p at 6 μg/kg/hr were based on data that contained some concentrations below quantitation level (BQL), and are therefore less reliable than the estimates at higher infusion rates. Cl_p was not affected by age ($p>0.4$).

11.5.2. Part 1 Pharmacokinetic Conclusions

- Pharmacokinetics regardless of age are linear across the dosing range of 12 μg/kg/hr to 36 μg/kg/hr.
- There were no statistically significant differences between the body weight-normalized Activated Protein C Cl_p values across all three age groups ($p>0.69$).

11.5.3. Dosing Regimen for Part 2 of Study F1K-MC-EVAO

Based on the overall consistency of body-weight adjusted Activated Protein C Cl_p of approximately 0.57 L/hr/kg in Part 1 of this study, all age groups received 24 μg/kg/hr for up to 96 hours.

11.5.4. Part 2 Preliminary Results

Pharmacokinetic results are available for 43 patients: 8 patients in the newborn to <1 age group, 25 patients in the 1 to <8 age group, and 10 patients in the 8 to <18 age group.

Table 11.7 contains a summary of the preliminary pharmacokinetic data in pediatric patients who received a 96-hour infusion of 24 μg/kg/hr drotrecogin alfa (activated).

Table 11.7. Mean Activated Protein C Plasma Steady-State Concentrations and Body Weight-Normalized Clearance

	Mean (CV)	
Pediatric Age Group	C_{ss} (ng/mL)	Cl_p (L/hr/kg)
0 years to <1 year N=8	65.1 (92.2)	0.513 (35)
≥1 year to <8 years N=25	56.6 (50.0)	0.549 (37.7)
≥8 years to <18 years N=10	92.7 (27.2)	0.323 (30.1)
Pediatric Patients 0 years to <18 years N=43	66.6 (56.6)	0.490 (41.2)
Adult Patients [a] N=326	51.5[b] NA	0.563 (46%)

Abbreviations: Clp = plasma clearance; Css = average observed steady-state APC plasma concentration; CV = coefficient of variation; N = number of patients.

[a] From Phase 3 study F1K-MC-EVAD, listed for purposes of comparison.

[b] CV was not reported in Study F1K-MC-EVAD.

The pharmacokinetic results are generally consistent with those in Part 1. The results in the oldest age group deviate somewhat from the observed data in the younger patients and also from the observations previously collected in Part 1 of the study.

Activated Protein C does not accumulate during the 96-hour infusion of drotrecogin alfa (activated) in the pediatric population with severe sepsis.

The Activated Protein C plasma concentrations in Part 2 of this study were collected up to 2 hours post-infusion. The measurable Activated Protein C plasma concentrations from all patients, regardless of age, were pooled. This approach is justified because drotrecogin alfa (activated) is dosed by body weight and no major age effects on the pharmacokinetics of drotrecogin alfa (activated) were observed in Part 1 of the study (Figure 11.1). The pooled estimate of half-life was 0.91 hours. Rapid disappearance of Activated Protein C from plasma is consistent with the observations in the adult patients

with severe sepsis (Study F1K-MC-EVAD), indicating a relatively short Activated Protein C half-life both in adult and pediatric sepsis populations.

11.5.5. Comparison of Pharmacokinetics of Drotrecogin Alfa (Activated) in Pediatric and Adult Patients with Severe Sepsis

The mean plasma Activated Protein C C_{ss} and body weight-adjusted Activated Protein C Cl_p in pediatric patients are consistent with those in adult patients with severe sepsis. Figure 11.2 shows the Cl_p plotted versus age for the adult and pediatric populations combined. Figure 11.3 shows the Cl_p versus body weight for the adult and pediatric populations combined.

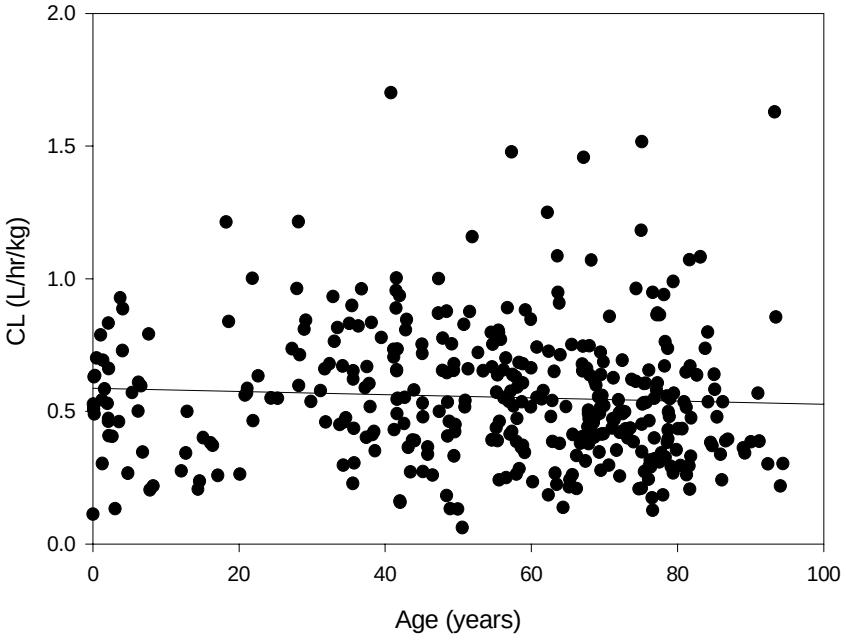


Figure 11.2. **Clearance versus age in adult and pediatric sepsis patients. Solid line derived from unweighted linear regression.**

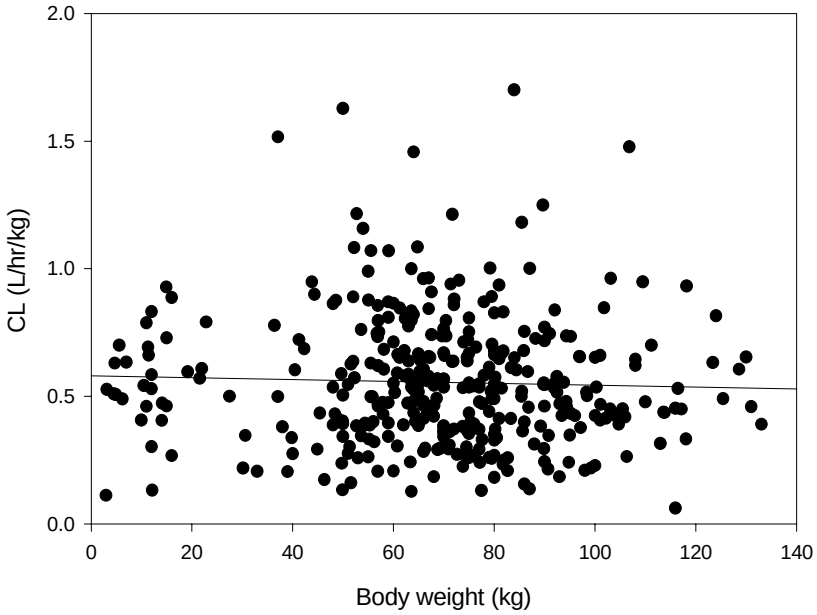


Figure 11.3. **Clearance versus body weight in adult and pediatric sepsis patients. Solid line derived from unweighted linear regression.**

These two figures show that Cl_p is comparable across all age groups and body weights.

11.6. Pharmacodynamic Results, Patients Enrolled in Part 2

In order to evaluate the similarity of the pharmacodynamic effects of drotrecogin alfa (activated) between children and adults, Part 2 of the pediatric protocol was prospectively designed to include the measurement of the following biomarkers: Protein C, D-dimer, and antithrombin. The following observations were based on the results of each one of these markers in both the pediatric and adult studies. The infusion rate and duration of the administration of drotrecogin alfa (activated) were the same for adult patients and pediatric patients in Part 2 of the pediatric study (24 μg/kg/hr for 96 hours).

The changes in Protein C activity levels, D-dimer levels, and antithrombin activity levels were similar in all pediatric age groups and were similar to the changes observed for adult patients with severe sepsis who were treated with drotrecogin alfa (activated). Table 11.8 contains a comparison of Protein C, D-dimer, and antithrombin results between pediatric patients and adult patients with severe sepsis.

Table 11.8. Comparison of Pharmacodynamic Observations in Pediatric and Adult Patients with Severe Sepsis Studies F1K-MC-EVAO and F1K-MC-EVAD

Pediatric Patients Part 2 Study F1K-MC-EVAO	Adult Patients Study F1K-MC-EVAD
Protein C Activity	
83.1% of patients with baseline values had values that were below the lower limit of normal for their age.	87.6% of patients with baseline values had values below the lower limit of normal.
The median percent increase in Protein C activity from baseline to the end of infusion was: 85% in the 0 to <1-year-old group (N=16) 54% in the 1 to <8-years-old group (N=25) 118% in the 8 to <18-years-old group (N=16)	The median percent increase in Protein C activity from baseline to Study Day 4 was: 43% in drotrecogin alfa (activated)-treated patients (N=777) 18% in placebo-treated patients (N=746)
D-Dimer Level	
100% of patients with baseline values had values that were above the upper limit of normal for their age.	99.7% of patients with baseline values had values above the upper limit of normal.
The median percent decrease in D-dimer levels from baseline to the end of infusion was: 33% in the 0 to <1-year-old group (N=16) 23% in the 1 to <8-years-old group (N=25) 5% in the 8 to <18-years-old group (N=15)	The median percent change in D-dimer levels from baseline to Study Day 4 was: 27% decrease in drotrecogin alfa (activated)-treated patients (N=770) 7% increase in placebo-treated patients (N=729)
Antithrombin Activity	
65.5% of patients had values that were below the lower limit of normal for their age.	81.7% of patients with baseline values had values below the lower limit of normal.
The median percent increase in antithrombin activity from baseline to the end of infusion was: 28% 0 to <1-year-old group (N=16) 33% 1 to <8-years-old group (N=25) 17% 8 to <18-years-old group (N=15)	The median percent increase in antithrombin activity from baseline to Study Day 4 was: 17% in drotrecogin alfa (activated)-treated patients (N=772) 15% in placebo-treated patients (N=734)

Source: LB10326#, CM10126#, CM10226#, CM10326#, Table EVAD.11.64, Table EVAD.14.97, Table EVAD.14.85, Table EVAD.14.99.

Analysis of the changes in plasma D-dimer concentrations for pediatric and adult patients with severe sepsis demonstrate that drotrecogin alfa (activated) has a similar beneficial effect for pediatric patients with severe sepsis compared to adult patients with severe sepsis.

11.7. Safety Summary (Parts 1 and 2)

Unlike in Study F1K-MC-EVAD, where clinical outcomes of organ failure are not reported as adverse events, all organ failures occurring in Study F1K-MC-EVAO were reported as adverse events. The duration of Study F1K-MC-EVAO was 14 days, as opposed to 28 days for Study F1K-MC-EVAD. Twenty of the 83 pediatric patients enrolled experienced serious adverse events, including those events, which resulted in death, were serious bleeding events, or adverse events that were considered serious for other reasons. Of these 20 patients with serious adverse events reported, only one event was considered to be possibly related to drotrecogin alfa (activated).

11.7.1. Deaths

Table 11.9 lists all deaths reported during the study. Eight deaths were reported, with only one death considered by the investigator to be causally related to drotrecogin alfa (activated). This patient experienced the serious adverse events of anisocoria and intracranial hemorrhage and the cause of death was reported as cerebral hemorrhage and edema. The narrative for this patient is presented below. All other deaths reported during the 14-day study period were reported to be due to sepsis-related complications.

- A 14-year-old patient admitted with severe multiorgan dysfunction, coagulopathy (platelet count = 71,000; APTT = 200.7 sec; and PT = 22.8 sec), and meningococcemia received drotrecogin alfa (activated). The infusion was discontinued after 10.5 hours due to anisocoria. Heparin was initiated for CVVHD the following day and continued until death. A technetium 99 scan on Study Day 13 confirmed clinical brain death. A CT scan revealed a frontal right parenchymal hematoma (intracranial hemorrhage) of uncertain age, but estimated to be 1 week old, severe cerebral edema, and a diffuse subarachnoid hemorrhage (intracranial hemorrhage).

Table 11.9. Listing of Deaths Part 1 and Part 2 Study F1K-MC-EVAO

Study Part	Age	Serious Adverse Event (COSTART Term)	Reported Cause of Death (Study Day)	Reported Causal Relationship to Drug
Part 2	3 yr	Heart Arrest, Shock, Peripheral Vascular Disorder	Multisystem organ failure due to shock (Study Day 3)	No
Part 2	8 yr	Prothrombin decreased, Thromboplastin decreased, Encephalopathy	Cerebral edema (Study Day 7)	No
Part 2	14 yr	Intracranial hemorrhage, anisocoria	Cerebral hemorrhage and edema (Study Day 13)	Possible
Part 2	3 mon	Shock	Multisystem organ failure (Study Day 2)	No
Part 2	13 yr	Heart arrest	Cardiac arrhythmia (Study Day 1)	No
Part 2	7 yr	Hypotension, Shock	Cardiogenic failure (Study Day 2)	No
Part 2	5 mon	Cerebral hemorrhage, Apnea, Meningitis	Pneumococcal meningitis (Study Day 9)	No
Part 2	1 mon	Shock, Sepsis	Overwhelming sepsis and multi-organ failure (Study Day 1)	No

Source: Clintrace.

11.7.2. Bleeding Events Reported as Serious Adverse Events

Table 11.10 provides a listing of patients with bleeding events reported as serious adverse events. Three patients (3/83, 3.6%) had serious bleeding events reported during the study drug infusion period. These events were nasopharyngeal hemorrhage, intracranial hemorrhage, and petechial cerebral hemorrhage. There was one additional serious bleeding event reported during the remaining study period (gastrointestinal hemorrhage). Narratives for these patients are presented below, except for the patient with intracranial hemorrhage whose clinical history is described in Section 11.6.1.

- A 6-year-old patient with septic shock, disseminated intravascular coagulation, and cardiovascular failure received the complete dose escalation of drotrecogin alfa (activated) over 4 days. On Study Day 2, approximately 1 hour after infusion was completed, multiple attempts were made to place a weighted nasogastric feeding tube without success. Shortly thereafter, the patient experienced acute bleeding from the nasopharyngeal region (nasopharyngeal hemorrhage), requiring 1500 mL of blood products. By Study Day 3 the bleeding had resolved clinically. The investigator attributed the bleeding episode to the patient's condition.
- A 5.5-month-old patient received 88 hours of drotrecogin alfa (activated) infusion for severe sepsis presumably caused by pneumococcal meningitis. The patient had a Glasgow Coma Scale score of 4, unequal pupils on admission, and received heparin for catheter patency prior to administration of drotrecogin alfa (activated). A CT scan performed on Study Day 4 revealed a petechial hemorrhage of the left frontal lobe (cerebral hemorrhage) believed by the investigator to be related to the patient's illness and not to the drotrecogin alfa (activated) infusion.
- A 15-year-old patient was enrolled with meningococcal septicemia and received 96 hours of drotrecogin alfa (activated). Following drotrecogin alfa (activated) infusion, heparin was used to maintain the hemofiltration circuit. The patient continued to require platelet transfusions after discontinuation of drotrecogin alfa (activated) for low platelet count. Approximately 52 hours following completion of study drug infusion frank blood was aspirated from the nasogastric tube (gastrointestinal hemorrhage). The investigator considered the bleeding to be unrelated to drotrecogin alfa (activated).

Table 11.10. Bleeding Events Reported as Serious Adverse Events Part 1 and Part 2 Study F1K-MC-EVAO

Study Part	Age	Serious Adverse Event (COSTART Term)	Fatal Outcome[a]	Reported Causal Relationship to Drug
Part 1	6 yr	Gangrene, Nasopharyngeal Hemorrhage	No	No
Part 2	14 yr	Anisocoria, Intracranial Hemorrhage	Yes	Possibly
Part 2	5 mon	Cerebral Hemorrhage, Apnea, Meningitis	Yes	No
Part 2	15 yr	Gastric bleeding, Cardiac arrest	No	No

[a] Events that resulted in a fatal outcome are also listed in Table 11.9.
Source: Clintrace.

11.7.3. Comparison of Bleeding Events and Mortality for Pediatric and Adult Patients with Severe Sepsis

Table 11.11 contains a comparison of the safety profile of drotrecogin alfa (activated) in pediatric patients and adult patients with severe sepsis.

The incidence of serious bleeding events in the pediatric population appear to be similar to those in the adult patient population. Two serious adverse events involving cerebral hemorrhage have been reported in Study F1K-MC-EVAO (one hematoma and one petechial hemorrhage); this may reflect the relatively high incidence of CNS infection in this population (Table 11.1) where petechial hemorrhage is a recognized complication of meningitis. The mortality rate for the pediatric patient population appears lower than that for the adult population from Study F1K-MC-EVAD. This comparison also supports the similar safety profile of the drug in pediatric patients versus adult patients.

Table 11.11. Comparison of Serious Bleeding Events and Deaths for Pediatric and Adult Patients with Severe Sepsis Studies F1K-MC-EVAO and F1K-MC-EVAD

Pediatric Patients Parts 1 and 2 Combined F1K-MC-EVAO	Adult Patients F1K-MC-EVAD
Bleeding Events Reported as Serious Adverse Events	
3 patients out of 83 (3.6%) experienced a bleeding event reported as a serious adverse event during the study drug infusion period (end of infusion plus 1 day).	20 of 850 (2.4%) drotrecogin alfa (activated) patients and 8 of 840 (1.0%) placebo patients experienced a bleeding event reported as a serious adverse event during the study drug infusion period.
4 patients out of 83 (4.8%) experienced a bleeding event reported as a serious adverse event during the 14-day study period.	30 of 850 (3.5%) drotrecogin alfa (activated) patients and 17 of 840 (2.0%) placebo patients experienced a bleeding event reported as a serious adverse event during the 28-day study period.
14-Day Mortality	
9.6% of patients died (8 of 83).	19.5% of drotrecogin alfa (activated) patients died (166 of 850). 23.9% of placebo patients died (201 of 840).

Source: Clintrace, Table EVAD.12.3, Table EVAD.11.38.

11.7.4. Bleeding Events Reported as Treatment-Emergent Adverse Events

Seventeen out of 83 (20.5%) patients experienced at least one bleeding event that was reported as a treatment-emergent adverse event. The majority of these treatment-emergent bleeding events were reported to be mild to moderate in severity. These events included minor oozing and bruising near incision and catheter insertion sites, hematuria, epistaxis, and blood tinged secretions from mucous membranes.

11.7.5. Treatment-Emergent Adverse Events

Seventy-eight out of 83 patients (94.0%) experienced at least one treatment-emergent adverse event. The most frequent adverse events reported were the following: fever, reported by 16 patients (19.3%); generalized edema, reported by13 patients (15.7%); and agitation, lung edema, and thrombocytopenia, which were each reported by 12 patients (14.5%).

11.7.6. Serious Adverse Events

Table 11.12 provides data on the 10 patients who experienced nonbleeding serious adverse events. All of these events were judged by the investigator to be unrelated to drotrecogin alfa (activated) administration.

Table 11.12. Listing of Other Serious Adverse Events Part 1 and Part 2 Study F1K-MC-EVAO

Study Part Enrollment	Age	Serious Adverse Event (COSTART Term)	Fatal Outcome	Reported Causal Relationship to Drug
Part 1	8 mon	Cerebral Ischemia	No	No
Part 1	7 day	Intestinal Perforation, Abscess	No	No
Part 1	1 mon	Respiratory Distress Syndrome	No	No
Part 1	14 yr	Choreoathetosis, Apnea	No	No
Part 1	7 yr	Necrosis	No	No
Part 2	1 yr	Peripheral Vascular Disorder	No	No
Part 2	1 yr	Shock	No	No
Part 2	12 yr	Hypotension, Apnea	No	No
Part 2	3 yr	Bradycardia	No	No
Part 2	4 yr	Purpura	No	No

Source: Clintrace.

11.8. Other Pediatric Experience

Additional safety data on pediatric patients with severe sepsis and purpura fulminans are currently being collected from ongoing studies. These studies include a compassionate use program for patients with purpura fulminans (Study F1K-MC-EVAS), a treatment use program for patients with severe sepsis (Study F1K-MC-EVBC), and the open-label studies F1K-MC-EVBE, F1K-MC-EVBF (conducted outside of the United States), and F1K-MC-EVBG (conducted outside of the United States) for patients with severe sepsis. Table 11.13 presents a summary of enrollment and safety data collected as of 29 May 2001.

Table 11.13. Summary of Other Pediatric Experience Enrollment through 29 May 2001

Trial	Study Design	Enrollment (0 < 18 years)	Status	Adverse Events
F1K-MC-EVAS	Compassionate use for purpura fulminans	14 (8 months to 17 years)	Enrollment Complete	Deaths: 1 death reported as clinical outcome Serious adverse events: 1 Bleeding events reported as serious adverse events: 0
F1K MC-EVBC	Treatment use for severe sepsis	20 (4 months to 12 years)	Ongoing	Deaths: 4 deaths, 3 were reported as clinical outcomes, 1 was intracranial infarct (none related to study drug) Serious adverse events: 1 Bleeding events reported as serious adverse events: 0
F1K-MC-EVBE F1K-MC-EVBF F1K-MC-EVBG	Open-label safety study for severe sepsis	4 (8 months to 4 years)	Ongoing	Deaths: 0 Serious adverse events: 0 Bleeding events reported as serious: 0

Source: Clintrace.

Among the 38 pediatric patients enrolled in the above studies, there were no bleeding events reported as serious adverse events.

11.9. Conclusions

- Baseline data describing the disease process for pediatric 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.
- Clearance of drotrecogin alfa (activated) is similar in pediatric and adult patients with severe sepsis. Steady-state concentrations of drotrecogin alfa (activated) were also similar in pediatric and adult patients with severe sepsis (66.6 ng/mL and 51.5 ng/mL, respectively).
- The pharmacodynamics of drotrecogin alfa (activated) are similar in pediatric and adult patients with severe sepsis.
- The safety profile of drotrecogin alfa (activated) appears to be similar in pediatric and adult patients with severe sepsis.
- The objectives of the pediatric study agreed upon by the Agency (Section 11.1) have been met. The results of the pediatric study support the indication of drotrecogin alfa (activated) in pediatric patients with severe sepsis.

12. Conclusions

The results of the Phase 2 and Phase 3 studies in patients with severe sepsis indicate that drotrecogin alfa (activated) substantially reduces mortality and is associated with a favorable benefit-risk profile. These data demonstrate that, in the population studied, 1 additional life would be saved for every 16 patients treated with drotrecogin alfa (activated).

There was a consistent effect of drotrecogin alfa (activated) on the relative risk of death across subgroups defined by baseline patient demographics, infection site, infection type, and clinical and biochemical measures of disease severity. These data indicate that critically ill patients with known or suspected infection and associated acute organ dysfunction may benefit from the use of drotrecogin alfa (activated) as adjunctive therapy to best standard of care.

However, there are instances where the potential bleeding risk associated with drotrecogin alfa (activated) administration may contraindicate its administration to patients with severe sepsis. These instances are predominately confined to clinical situations where bleeding would be associated with a high risk of death or significant morbidity. These instances include the following:

- Patients with active internal bleeding
- Patients with a history of recent (within 3 months) hemorrhagic stroke
- Patients with a history of recent (within 2 months) intracranial or intraspinal surgery, or severe head trauma requiring hospitalization
- Trauma patients at increased risk of life-threatening bleeding
- Patients with an epidural catheter in place
- Patients with an intracranial neoplasm or mass lesion

Additionally, there are instances when the potential bleeding risk associated with drotrecogin alfa (activated) administration should cause the physician to use extreme caution when administering drotrecogin alfa (activated). These instances are predominately confined to clinical situations where the bleeding risk associated with drotrecogin alfa (activated) may be increased or where a bleeding event would be associated with significant morbidity. These instances include the following:

- Concurrent heparin therapy ≥15 international units/kg/hr
- Platelet count $<30{,}000/mm^3$
- Recent (within 6 weeks) gastrointestinal bleeding
- Recent administration (within 3 days) of thrombolytic therapy

- Recent administration (within 7 days) of oral anticoagulants or glycoprotein IIb/IIIa inhibitors
- Recent administration (within 7 days) of aspirin >650 mg per day or other platelet inhibitors
- Recent (within 3 months) ischemic stroke
- Patients with intracranial arterioveneous malformation or aneurysm
- Known bleeding diathesis except for acute coagulopathy related to sepsis
- Chronic severe hepatic disease
- Any other condition in which bleeding constitutes a significant hazard or would be particularly difficult to manage because of its location

Pharmacokinetic data indicate that drotrecogin alfa (activated) should be discontinued 2 hours prior to any invasive procedure. Based on treatment guidelines employed in the pivotal Phase 3 study F1K-MC-EVAD, drotrecogin alfa (activated) may be restarted following less invasive procedures (once hemostasis has been achieved). Drotrecogin alfa (activated) may be restarted 12 hours after major invasive procedures or surgery.

In conclusion, these data indicate that drotrecogin alfa (activated) administration is associated with a favorable benefit/risk profile.

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Appendix 1: APACHE II Scale

The APACHE II Severity of Disease Classification System

A Acute Physiology Points:

	High Abnormal Range				Low Abnormal Range				
Physiologic variable	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature (rectal, °C)	≥41	39-40.9		38.5-38.9	36- 38.4	34-35.9	32 -33.9	30 -31.9	≤29.9
Mean Arterial Pressure (mm Hg)	≥160	130 – 155	110-129		70-109		50-69		≤49
Heart rate (ventricular response)	≥ 180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory rate (non-ventilated orientation)	≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation: $AaDO_2$ or PaO_2 (mmHg) a. FIO_2 ≥0.5 record only AaDO b. FIO_2 <0.5 record only PaO_2	≥500	350-499	200-349		<200 PO_2 >70	PO_2 61-70		PO_2 55-60	PO_2 <55
Arterial pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Serum sodium (mMd/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum potassium (mMd/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
Serum creatinine (mg/100 mL) (double point score for acute renal failure.)	≥3.5	2-3.4	1.5-1.9		0.6-1.4		<0.6		
Hematocrit (%)	≥60		50-59.9	46-49.9	30-45.9		20-29.9		<20
White Blood Count	≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
Glasgow Coma Score (GCS) Score = 15 minus actual GCS									
Total Acute Physiology Score									
Serum HCO_3 (venus, mMd/L) (not preferred, use if no ABGs)	≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	<15

(continued)

B AGE POINTS:

Assign points to age as follows:

Age (yrs)	Points
≥ 44	0
45 – 54	2
56 – 64	3
65 – 74	5
≤ 75	6

C CHRONIC HEALTH POINTS:

If the patient has a history of severe organ insufficiency or is immunocompromised, assign points as follows:

a. nonoperative or emergency post-operative patients: 5 points

b. elective postoperative patients: 2 points

Definitions: Organ insufficiency or immunocompromised state evident prior to this hospital admission and conforming to the following criteria:
LIVER: Biopsy proven cirrhosis and documented portal hypertension; episodes of past upper GI bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma.
CARDIOVASCULAR: New York Heart Association Class IV.
RESPIRATORY: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, ie, unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40 mm Hg), or respirator dependency.
RENAL: Receiving chronic dialysis.
IMMUNOCOMPROMISED: Patient has received therapy that suppresses resistance to infection, eg, immunosuppression, chemotherapy, radiation, long term or recent high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection (eg, leukemia, lymphoma, AIDS)

APACHE II SCORE

Sum of A + B + C

A APS Points _____
B Age Points _____
C Chronic Health Points _____

TOTAL APACHE II _____

Appendix 2: SOFA Table of Organ Dysfunction

SOFA Table of Organ Dysfunction[a]

SOFA Score	1	2	3	4
Respiration PaO_2/FiO_2 mmHg	<400	<300	<200 with respiratory support	<100 with respiratory support
Coagulation Platelets $x10^3/mm^3$	<150	<100	<50	<20
Liver Bilirubin mg/dL (µmol/L)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (>204)
Cardiovascular Hypotension	MAP <70mmHg	Dopamine ≤5 or dobutamine (any dose)[b]	Dopamine >5 or epinephrine ≤ 0.1 or norepinephrine ≤ 0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Renal Creatinine, mg/dL (µmol/L) or urine output	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) or <500 mL/day	>5.0 (>440) or <200 mL/day

Abbreviations: MAP = mean arterial pressure; SOFA = Sequential Organ Failure Assessment.

[a] Adapted from Vincent JL et al. 1996. Reprinted with permission.

[b] Adrenergic agents administered for at least 1 hour (doses given are in µg/kg/min).

Appendix 3: Normal Biomarker Ranges

Normal Biomarker Reference Ranges

Age Group:	0 years to <1 year	≥1 year to <8 years	≥8 years to <18 years	Adult
Protein C (%)	>21	>51	>81	>81
D-dimer (µg/mL)	0 to 0.39	0 to 0.39	0 to 0.39	0 to 0.39
Antithrombin (%)	50 to 110	80 to 120	80 to 120	80 to 120

Source: Central Laboratory Reference Ranges.

Appendix 4: References from Literature Review of Pediatric Sepsis

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Appendix 5: Published Manuscript of Study F1K-MC-EVAD: Efficacy and Safety of Recombinant Human Activated Protein C for Severe Sepsis

Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber JE, Helterbrand JD, Ely EW, Fisher CJ Jr; Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. 2001. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 344(10):759-62.