# TREATMENT OF SEPSIS BY LOW DOSE ADMINISTRATION OF TISSUE FACTOR PATHWAY INHIBITOR (TFPI)

## CROSS-REFERENCE TO RELATED APPLICATION

[01] This application claims priority to provisional application Serial No. 60/328,806 filed October 15, 2001, hereby incorporated by reference in its entirety.

## FIELD OF THE INVENTION

[02] The present invention is a method for prophylactically and therapeutically treating sepsis, septic shock, and acute or chronic inflammation while minimizing adverse side effects. More specifically, it comprises administering low doses of a tissue factor pathway inhibitor protein to attenuate amplified or activated physiological pathways associated with sepsis and septic shock.

## BACKGROUND OF THE INVENTION

[03] Sepsis and its sequela septic shock remain among the most dreaded complications after surgery and in critically ill patients. The Center for Disease Control has ranked septicemia as the 13th leading cause of death in the United States (MMWR, 1987, 39:31 and US Dept. of Health and Human Services, 37:7, 1989), and the 10th leading cause of death among elderly Americans (*see* MMWR, 1987, 32:777). The incidence of these disorders is increasing, and mortality remains high. Caring for patients with septicemia was estimated to cost billions of dollars annually (MMWR, 1987, 39:31). Death can occur in 28% to 60% of the patients, and this percentage has not seen any sizeable improvement for more than 20 years. Gram-positive and gram-negative bacterial infections are equally likely to lead to sepsis and septic shock.

[04] Sepsis is a toxic condition resulting from the spread of various microorganisms including bacteria, fungi, parasites, viruses, and their products (*e.g.*, endotoxin of gram negative bacteria) from a focus of infection. Septicemia is a form of sepsis, and more particularly is a toxic condition resulting from invasion of the blood stream by bacterial products

from a focus of infection. Sepsis can result in shock in many ways, some related to the primary focus of infection and some related to the systemic effects of the bacterial endotoxins. For example, in septicemia, bacterial products, along with proinflammatory cytokines, such as IL-1, IL-6 and TNF, activate the coagulation system and initiate platelet aggregation. The process leads to exuberant systemic inflammation and widespread blood clotting or severe bleeding associated with clinical symptoms including a drop in blood pressure and finally kidney, heart and lung failure.

[05] Septic shock is characterized by inadequate tissue perfusion, leading to insufficient oxygen supply to tissues, hypotension and oliguria. Septic shock occurs because microorganisms including bacteria and their products, such as LPS, directly activate the immune system and other host defense mechanisms including coagulation, and the complement system that in turn amplify each other through a series of cross-talk mechanisms. The over-amplification of these host defense mechanisms ultimately results in cell injury and altered blood flow in the microvasculature leading to multiple organ failure. Microorganisms frequently activate the classic complement pathway, and endotoxin activates the alternate pathway. Complement activation, leukotriene generation and the direct effects of endotoxin on neutrophils lead to accumulation of these inflammatory cells in the lungs, release of the enzymes and production of toxic oxygen radicals which damage the pulmonary endothelium and initiate the acute respiratory distress syndrome (ARDS). ARDS is a major cause of death in patients with septic shock and is characterized by pulmonary congestion, granulocyte aggregation, hemorrhage, and capillary thrombi.

[06] Fulminating disseminated intravascular coagulation (DIC), characterized by excessive clotting or severe bleeding, has been found to occur in up to 80% of septic patients, and coagulation abnormalities of all types are more frequent (Levi *et al.*, Thromb. Haemost. 82:695-705, 1999). Coagulopathies can ultimately lead to multiple organ failure, which is a major immediate cause of death in severe sepsis patients. DIC is a coagulopathic disorder that occurs in response to invading microorganisms characterized by widespread deposition of fibrin in small vessels. The initiating cause of DIC appears to be the release

of thromboplastin (tissue factor) into the circulation. During this process, there is a reduction in fibrinogen and platelets, and a rise in fibrin split products resulting in fibrin deposition in blood vessels. The patients either suffer from thrombosis or hemorrhage depending on the extent of exhaustion of the coagulation protease inhibitors during the disease process. Some common complications of DIC are severe clinical bleeding, thrombosis, tissue ischaemia and necrosis, hemolysis and organ failure. Part of the regulation of the coagulation cascade depends on the rate of blood flow. When flow is decreased, as it is in DIC and sepsis, the problems are magnified. DIC (clinically mild to severe form) is thought to occur with high frequency in septic shock patients and several other syndromes such as head trauma and burns, obstetric complications, transfusion reactions, and cancer.

**[07]** DIC is associated with a variety of inflammatory conditions. In septic patients, DIC and acute respiratory distress syndrome (ARDS) were the variables most predictive of death by day 7 (risk ratios 4 and 2.3). (Martin *et al.*, 1989, Natural History in the 1980s, Abstract No. 317, ICAAC Meeting, Dallas). The cascade of events that leads to sepsis, including expression of tissue factor on the endothelium or exposure of tissue factor to the blood and the release of tissue factor into the circulation, is very complex. Various cytokines are released from activated monocytes, endothelial cells, and others. These cytokines include tumor necrosis factor (TNF) interleukin 1 (IL-1) (which are known to up-regulate tissue factor expression), interleukin 6 (IL-6), gamma interferon (IFNγ), interleukin 8 (IL-8), and others. The complement cascade also is activated as demonstrated by the rise in C3a and C5a levels in the plasma of septic patients.

**[08]** Tissue factor pathway inhibitor (TFPI) is a serine protease inhibitor present in mammalian blood plasma. Thomas, *Bull. Johns Hopkins Hosp. 81*, 26 (1947); Schneider, *Am. J. Physiol. 149*, 123 (1947); Broze & Miletich, *Proc. Natl. Acad. Sci. USA 84,* 1886 (1987). TFPI is also known as tissue factor inhibitor, tissue thromboplastin inhibitor, Factor III inhibitor, extrinsic pathway inhibitor (EPI), and lipoprotein-associated coagulation inhibitor (LACI). The name "tissue factor pathway inhibitor" (TFPI) was accepted by the International Society on Thrombosis and Hemostasis on June 30, 1991.

[09] Blood coagulation activation is the conversion of fluid blood to a solid gel or clot. In addition, consumption of the coagulation proteases leads to excessive bleeding. The main event is the conversion of soluble fibrinogen to insoluble strands of fibrin, although fibrin itself forms only 0.15% of the total blood clot. This conversion is the last step in a complex enzyme cascade. The components (factors) are present as zymogens, inactive precursors of proteolytic enzymes, which are converted into active enzymes by proteolytic cleavage at specific sites. Activation of a small amount of one factor catalyzes the formation of larger amounts of the next, and so on, resulting in an amplification that results in an extremely rapid formation of fibrin.

[10] Coagulation is believed to be initiated by vessel damage which exposes factor VIIa to tissue factor (TF), which is expressed on cells beneath the endothelium. The factor VIIa-TF complex cleaves factor X to factor Xa and cleaves factor IX to factor IXa. TFPI binds to both factor VIIa and factor Xa. The complex formed between TFPI, factor VIIa (with its bound TF), and factor Xa inhibits further formation of factors Xa and Ixa, required for sustained hemostasis. Broze, Jr., Ann. Rev. Med. 46:103 (1995).

[11] Activation of the coagulation cascade by bacterial endotoxins introduced directly into the bloodstream can result in extensive fibrin deposition on arterial surfaces, as well as depletion of fibrinogen, prothrombin, factors V and VIII, and platelets. In addition, the fibrinolytic system is stimulated, resulting in further formation of fibrin degradation products.

[12] At the same time as coagulation activation is apparently initiated by bacterial products (*e.g.*, endotoxin), contravening mechanisms also appear to be activated by clotting, namely activation of the fibrinolytic system. Activated Factor XIII converts plasminogen pro-activator, to plasminogen activator that subsequently converts plasminogen to plasmin, thereby mediating clot lysis. The activation of plasma fibrinolytic systems may therefore also contribute to bleeding tendencies.

[13] Endotoxemia is associated with an increase in the circulating levels of tissue plasminogen activator inhibitor (PAI). This inhibitor rapidly inactivates tissue plasminogen activator

(TPA), thereby hindering its ability to promote fibrinolysis through activation of plasminogen to plasmin. Impairment of fibrinolysis may cause fibrin deposition in blood vessels, thus contributing to the DIC associated with septic shock.

[14] Efforts are ongoing to identify satisfactory interventions for the prevention or treatment of sepsis and associated coagulopathies. An agent that interrupts the coagulation pathway is not necessarily effective as a therapeutic or a prophylactic treatment of septic shock. For example, heparin is a commonly used anticoagulant. However, management of the use of heparin has been difficult because heparin can induce excessive bleeding or attenuate coagulation without evidence of a survival benefit. See Aoki *et al.*," A Comparative Double-BLIND randomized Trial of Activated Protein C and Unfractionated Heparin in the Treatment of Disseminated Intravascular Coagulation," *Int. J. Hematol. 75*, 540-47 (2002). Several clinical trials, mainly in meningococcal endotoxemia where fulminating DIC is a prominent feature, have failed to demonstrate reduction of mortality in sepsis by heparin treatment. *See*, for example, Corrigan *et al.*, "Heparin Therapy in Septacemia with Disseminated Intravascular Coagulation. Effect on Mortality and on Correction of Hemostatic Defects," N. Engl. J. Med., 283:778-782 (1970); Lasch *et al.*, Heparin Therapy of Diffuse Intravascular Coagulation (DIC)", Thrombos. Diathes. Haemorrh., 33:105 (1974); Straub, "A Case Against Heparin Therapy of Intravascular Coagulation", Thrombos. Diathes. Haemorrh., 33:107 (1974).

[15] Due to its known abilities to attenuate activation of the coagulation cascade and ameliorate the inflammatory response and bind endotoxin, TFPI has been proposed as a drug useful for treating sepsis. Administration of recombinant human ala-TFPI (a TFPI analog) has been shown to improve survival rates in animal models of sepsis. *See, e.g.*, U.S. Patent No. 6,063,764. As an endogenous protein, TFPI is well tolerated. TFPI administration by intravenous infusion or subcutaneous injection has been shown to reduce clotting ability, which is manifested as increased prothrombin time (PT). In studies of animals and humans, prolongations of PT were linearly related to the increase of plasma TFPI. A.A. Creasey, Sepsis 3:173 (1999).

[16] There remains a need in the art for treatment approaches that will inhibit the lethal effects of sepsis and simultaneously minimize potentially serious side effects.

## SUMMARY OF THE INVENTION

[17] One embodiment of the present invention is a method of treating sepsis comprising administering TFPI or a TFPI analog to a patient who has sepsis, or who is at risk of becoming septic, by continuous intravenous infusion at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period of at least about 72 hours.

[18] Another embodiment of the present invention is a prophylactic method for decreasing the risk and severity of sepsis comprising administering TFPI or a TFPI analog to a patient susceptible to sepsis or suspected of being septic by continuous intravenous infusion at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period of at least about 72 hours.

[19] Another embodiment of the present invention is a method for prophylactically and therapeutically treating acute inflammation, including sepsis and septic shock comprising administering to a patient (i) a continuous intravenous infusion of TFPI or a TFPI analog at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr and (ii) an additional agent selected from the group consisting of an antibiotic, a monoclonal antibody, a cytokine inhibitor, and a complement inhibitor.

[20] Another embodiment of the present invention is a method for treating a disease state not associated with DIC and in which TNF, IL-1, or another cytokine up-regulates tissue factor expression, comprising administering to a patient a continuous intravenous infusion of an agent selected from the group consisting of TFPI or a TFPI analog at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period of at least about 72 hours. In a preferred embodiment, the disease state is chronic or acute inflammation. In another

preferred embodiment, said patient has a plasma concentration of IL-6 that decreases during said administration period.

[21] Other embodiments include any of the above embodiments wherein said TFPI analog is non-glycosylated ala-TFPI.

[22] Other embodiments include any of the above embodiments wherein said TFPI analog comprises a first Kunitz domain consisting of amino acids 19-89 of SEQ ID NO:1. In a preferred embodiment, said TFPI analog further comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

[23] Other embodiments include any of the above embodiments wherein said TFPI analog comprises amino acids 1-160 of SEQ ID NO:1 or wherein said TFPI analog comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

[24] Other embodiments include any of the above embodiments wherein said dose rate is equivalent to administration of reference ala-TFPI at a dose rate from about 0.010 to about 0.045 mg/kg/hr. In a preferred embodiment, said dose rate is equivalent to administration of reference ala-TFPI at a dose rate of about 0.025 mg/kg/hr.

[25] Other embodiments include any of the above embodiments wherein said administration period is at least about 96 hours.

[26] Other embodiments include any of the above embodiments wherein said dose rate is administered to provide a total dose equivalent to administration of reference ala-TFPI at a total dose from about 0.024 to about 4.8 mg/kg.

[27] Other embodiments include any of the above embodiments wherein said dose rate is administered to provide a daily dose equivalent to administration of reference ala-TFPI at a daily dose of at least about 0.006 mg/kg and less than about 1.2 mg/kg.

[28] Other embodiments include any of the above embodiments wherein said TFPI or said TFPI analog is administered to a patient having a baseline International Normalized Ratio (INR) of at least about 1.2.

[29] Other embodiments include any of the above embodiments, further comprising terminating administering said TFPI or TFPI analog when said patient has an INR either exceeding a baseline INR by at least 20% or having a value of at least about 2.5.

[30] Other embodiments include any of the above embodiments wherein said patient has an APACHE II score of at least 20.

[31] Other embodiments include any of the above embodiments wherein said patient has a baseline plasma IL-6 concentration of at least about 1000 pg/ml.

[32] Other embodiments include any of the above embodiments wherein said patient is suffering from shock.

[33] Other embodiments include any of the above embodiments wherein said patient is suffering from ARDS.

[34] Other embodiments include any of the above embodiments wherein said patient has a pulmonary score, an ICU score, or a multiple organ dysfunction score that increases during said administration period.

[35] Other embodiments include any of the above embodiments wherein said TFPI or TFPI analog is prepared from a lyophilized composition comprising TFPI or a TFPI analog.

[36] Other embodiments include any of the above embodiments wherein said TFPI or TFPI analog is administered as a formulation comprising arginine.

[37] Other embodiments include any of the above embodiments wherein said TFPI or TFPI analog is administered as a formulation comprising citrate.

[38] Other embodiments include any of the above embodiments wherein said TFPI or TFPI analog has a concentration of about 0.15 mg/ml in a formulation comprising about 300 mM arginine hydrochloride and about 20 mM sodium citrate and having a pH of about 5.5.

[39] Other embodiments include any of the above embodiments, further comprising administering, during or within 24 hours of said administration period, an additional agent selected from the group consisting of an antibiotic, an antibody, an endotoxin antagonist, a tissue factor analog having anticoagulant activity, an immunostimulant, a cell adhesion blocker, heparin, BPI protein, an IL-1 antagonist, pafase (PAF enzyme inhibitor), a TNF inhibitor, an IL-6 inhibitor, and an inhibitor of complement. In a preferred embodiment, said additional agent is an antibody, wherein said antibody binds specifically to an antigen selected from the group consisting of TNF, IL-6, and M-CSF.

[40] Further embodiments of the present invention are apparent in view of the below-referenced drawings in conjunction with the detailed description.

## BRIEF DESCRIPTION OF THE DRAWINGS

[41] Figs. 1A and 1B show the effect of low dose ala-TFPI administration on survival (1A) and decrease of IL-6 levels (1B) in septic human patients. Fig. 1A shows Kaplan Meier survival curves for 210 septic patients receiving either placebo or recombinant non-glycosylated ala-TFPI expressed in *E. coli* by continuous intravenous infusion at either 0.025 or 0.05 mg/kg/hr (*see* Example 5 for details). The results show a trend toward reduction in mortality rate with ala-TFPI treatment. Fig. 1B shows the time for a decrease to 35% of baseline IL-6 levels in the same patients represented in Fig. 1A. Low dose ala-TFPI administration significantly decreased IL-6 levels compared to placebo using two different statistical approaches (M&M and LOCF; $p = 0.009$ and 0.025, respectively).

[42] Figs. 2A and 2B show the kinetics of thrombin-antithrombin (TATc) levels in the 210 septic patients described in Example 5. In Fig. 2A TATc levels are shown separately for

the 0.025 mg/kg/hr and 0.050 mg/kg/hr doses of ala-TFPI as well as for placebo administration. Fig. 2B shows the combined data for both 0.025 mg/kg/hr and 0.050 mg/kg/hr doses versus placebo. The geometric mean of TATc was significantly lower in the ala-TFPI treated versus placebo-treated patients within the first 24 hours of ala-TFPI administration. The TATc levels continued to be significantly lower throughout 72 hours.

[43] Fig. 3 shows the relationship between INR and plasma concentration of ala-TFPI ("rTFPI") in healthy human volunteers.

## DETAILED DESCRIPTION OF THE INVENTION

[44] Continuous low dosage administration of TFPI or analogs of TFPI (hereinafter "low dose TFPI administration") is effective in the prophylaxis and treatment of sepsis yet significantly minimizes potentially harmful complications found in some patients treated at higher doses. Low dose TFPI administration generally is carried out by continuous intravenous infusion of TFPI or an analog of TFPI at a dose rate equivalent to administration of reference ala-TFPI at a dose rate of at least about 0.00025 mg/kg/hr and less than about 0.050 mg/kg/hr.

[45] Low dose TFPI administration inhibits or attenuates acute or chronic inflammation, including sepsis and septic shock. When low dose TFPI administration is continued for at least three days, the risk of death from sepsis is reduced while the rate of complications from adverse side effects, particularly bleeding disorders, is minimized compared to administration of higher doses of TFPI.

[46] A further advantage of low dose TFPI administration is the avoidance of tolerance effects which, at sufficiently high doses, can reduce the plasma concentration of TFPI. Tolerance effects are stimulated half-maximally at a plasma TFPI concentration of about 850 ng/ml, whereas with low dose TFPI administration plasma levels generally stay below 500 ng/ml.

[47] The invention achieves the optimum balance between the effectiveness of TFPI and analogs of TFPI in treating inflammatory disorders such as sepsis and the avoidance of harmful side effects, which can result from higher doses or alternative strategies for administration. Low dose TFPI administration is also effective in the prophylaxis and treatment of sepsis-associated coagulation disorders such as disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), and multiple organ failure.

[48] Efficacy in treating sepsis and other inflammatory conditions is retained, and adverse side effects such as bleeding are minimized when TFPI or a TFPI analog is given at a dose rate equivalent to administration of reference ala-TFPI at a dose rate of at least about 0.00025 mg/kg/hr (0.00417 μg/kg/min) and less than about 0.050 mg/kg/hr (0.833 μg/kg/min). For optimum combined efficacy and safety, the dose rate is preferably equivalent to a dose rate of reference ala-TFPI of at least about 0.010 mg/kg/hr (0.167 μg/kg/min) and less than about 0.045 mg/kg/hr (0.833 μg/kg/min), or equivalent to a dose rate of reference ala-TFPI of at least about 0.020 mg/kg/hr and less than about 0.040 mg/kg/hr, and most preferably equivalent to a dose rate of reference ala-TFPI of about 0.025 mg/kg/hr (0.417 μg/kg/min)). The route of administration is generally by intravenous administration, with continuous intravenous infusion preferred. Infusion can be administered for at least about 72, 96, 120, or 240 hours. Preferably, continuous infusion is administered for 3 to 8 days, more preferably 3 to 6 days, and most preferably for about 4 days. To administer by continuous infusion means that the infusion is maintained at approximately the prescribed rate without substantial interruption for most of the prescribed duration. Alternatively, intermittent intravenous infusion can be used. If intermittent infusion is used, then a time-averaged dose rate should be used which is equivalent to the dose rates described above for continuous infusion. In addition, the program of intermittent infusion must result in a maximum serum concentration not more than about 20% above the maximum concentration obtained using infusion. For the avoidance of adverse reactions in the patient, particularly side effects involving bleeding, the dose rate should be less than a dose rate which is equivalent to continuous intravenous infusion of reference ala-TFPI at about 0.050 mg/kg/hr.

[49] All doses described herein, including dose rates and total doses, are subject to up to 10% variation in practice due to unavoidable imprecision in determining quantities such as protein concentration and biological activity with the prothrombin assay (see below). That is, any actually administered dose up to 10% higher or 10% lower than a dose stated herein is considered to be about the stated dose. For this reason, all doses have been stated as "about" a specific dose. For example, a dose described as "about 0.025 mg/kg/hr" is considered equivalent to any actual dose ranging from 0.0225 to 0.0275 mg/kg/hr.

[50] A bolus injection or a briefly higher infusion rate of TFPI or an analog of TFPI may also be employed in the practice of the present invention if followed by low dose TFPI administration. For example, a bolus injection or higher infusion rate can be used to reduce the equilibration time of administered TFPI or TFPI analog in the circulation of a patient. In doing so, the eventual steady state plasma level of TFPI can be reached more rapidly, and receptors for TFPI can be saturated faster. Administration of reference ala-TFPI to humans at about 0.025 mg/kg/hr for 2 hours increases plasma levels of TFPI (plus ala-TFPI) from about 80 ng/ml to about 125 ng/ml, or an increase of approximately 50%. The same level will be reached faster if the infusion rate is increased, or a bolus injection is used. Higher infusion rates will result in higher levels if infusion is continued until steady state is obtained. Steady state level for administration of reference ala-TFPI at about 0.050 mg/kg/hr is about 300 ng/ml, and for administration of reference ala-TFPI at about 0.33 or about 0.66 mg/kg/hr is at least 2 μg/ml in patients with sepsis.

[51] Total daily dose administered to a host in a single continuous infusion or in divided infusion doses may be in amounts, for example, equivalent to administration of at least about 0.006 mg/kg/day to less than about 1.2 mg/kg/day of reference ala-TFPI, more usually equivalent to administration of from about 0.24 mg/kg/day to less than about 1.2 mg/kg/day of reference ala-TFPI, and preferably equivalent to about 0.6 mg/kg/day of reference ala-TFPI. Lower amounts within this range may be useful for prophylactic or other purposes. The dosing protocols of the invention can also be expressed as the total dose administered to the patient. The total dose is the mathematical product of the rate of

infusion and the total time of infusion. For example, at the preferred dose rate of about 0.025 mg/kg/hr for reference ala-TFPI and the preferred infusion time of 96 hours, the total dose is about 2.4 mg reference ala-TFPI per kg body weight. The total dose of TFPI administered according to the invention is equivalent to at least about 0.75 μg/kg and less than about 4.8 mg/kg of reference ala-TFPI. Preferably the total dose is equivalent to at least about 1 mg/kg and less than about 4.8 mg/kg of reference ala-TFPI. More preferably the total dose is equivalent to about 2.4 mg/kg of reference ala-TFPI.

[52] One factor which can be used to adjust the dosage regimen is the individual patient's coagulation function, which is typically measured using a prothrombin time (PT) assay, or the International Normalized Ratio (INR). INR is the standardization of the PT assay in which the assay is calibrated against an international reference thromboplastin reagent. *See, e.g.*, R.S. Riley et al., J. Clin. Lab. Anal. 14:101-114 (2000). The INR response to ala-TFPI in healthy human volunteers was approximately linear over the range of plasma concentrations seen (Fig. 3). The overall change in INR was 1.2 units per 1 μg/ml increase of plasma ala-TFPI concentration. In a pharmacodynamic model based on combined data from healthy volunteers and sepsis subjects, the INR response to ala-TFPI was best described by a log-linear model in which log INR was linearly related to ala-TFPI plasma concentration. The log-linear nature of the response means that subjects with elevated INR at baseline (commonly seen in sepsis subjects) are likely to experience greater anticoagulant responses than subjects with low baseline values who have similar levels of circulating ala-TFPI. Furthermore, the slope of the concentration-response curve appeared to be higher in sepsis subjects than in healthy volunteers, suggesting that subjects with severe sepsis may have enhanced sensitivity to ala-TFPI. In that study, the ala-TFPI dose rates in the patient population ranged from a dose equivalent to reference ala-TFPI at about 0.025 mg/kg/hr to a dose equivalent to reference ala-TFPI at about 0.66 mg/kg/hr. Data from a phase II study of ala-TFPI in sepsis patients using a dose rate equivalent to reference ala-TFPI at about 0.025 or about 0.050 mg/kg/hr indicated a greater reduction of mortality by ala-TFPI in patients whose baseline INR was at least 1.2 (see Table 11 below). Thus, is some embodiments of the invention, low dose TFPI administration is carried out only with patients whose baseline INR prior to low dose

TFPI administration is at least 1.2, 1.25, 1.3, 1.4, 1.5, 1.6, 1.8, or 2.0. In other embodiments, low dose TFPI administration is reduced by lowering the dose rate of TFPI administration or is terminated altogether if the patient's INR increases over baseline by at least 10%, 15%, 20%, 25%, or 30% or more, or if the patient's INR reaches a dangerously high value such as at least about 2.3, 2.4, 2.5, 2.6, 2.7, 3.0, or 3.5.

[53] The dosing regimens described above, including dosing rate on a mg/kg/hr basis and total daily dose, are expressed as a dose "equivalent to administration of reference ala-TFPI." This means that they are determined quantitatively by normalization to a dose of "reference ala-TFPI" which is defined as mature, 100% pure (on a protein basis), properly folded, biologically active, non-glycosylated ala-TFPI. Ala-TFPI is an analog of TFPI whose amino acid sequence is depicted in SEQ ID NO:2. Other forms of TFPI can also be used in the invention, including mature, full-length TFPI and analogs thereof (see below). In order to determine the appropriate dosing range for practicing the invention with forms of TFPI other than ala-TFPI and with preparations of ala-TFPI or another TFPI analog that are less than 100% pure, the dosing ranges described herein for reference ala-TFPI can be adjusted based on the intrinsic biological activity of the particular form of TFPI and further adjusted based on the biochemical purity of the preparation. The intrinsic biological activity of TFPI or a TFPI analog refers to the specific activity, as defined by the prothrombin assay, of the mature, 100% pure, properly folded TFPI or TFPI analog. Thus, the equivalent dose is calculated as (reference ala-TFPI dose) / ((relative intrinsic activity) x (biochemical purity)), where relative intrinsic activity refers to (intrinsic activity of analog) / (intrinsic activity of reference ala-TFPI). For example, if a particular TFPI analog has an intrinsic biological activity which is 80% that of reference ala-TFPI, then the equivalent dose for the particular TFPI analog are obtained by dividing the dose values for reference ala-TFPI by 0.8. Further, if the formulation administered to a patient is, for example, only 90% biochemically pure, *i.e.*, comprising 10% of molecular species which lack biological activity of TFPI, then an additional correction of the reference dose values for ala-TFPI is performed by dividing the dose values by 0.9. Thus, for a hypothetical TFPI analog which has 80% of the intrinsic activity of ala-TFPI and is 90% biochemically pure as administered, a dose rate

equivalent to administration of reference ala-TFPI at 0.025 mg/kg/hr would be 0.0347 mg/kg/hr (*i.e.*, 0.025/(0.8 x 0.9)).

[54] Equivalent doses of a particular preparation can also be determined without knowing either intrinsic activity or biochemical purity by determining relative biological activity. Relative biological activity can be determined by comparing a particular preparation of a TFPI analog to a TFPI biological activity standard using the prothrombin time assay. For example, ala-TFPI produced according to the method of Example 9 of WO 96/40784, which contains about 85-90% biologically active ala-TFPI molecular species, can be used as a TFPI biological activity standard. Ala-TFPI produced according to the method of Example 9 of WO 96/40784 has about 85-90% of the activity of reference ala-TFPI in the prothrombin assay. In plotting a prothrombin time standard curve, the log of clotting time is plotted against the log of TFPI concentration. If the TFPI biological activity standard possesses 85-90% of the activity of reference ala-TFPI, then a standard curve can be prepared which is equivalent to that for reference ala-TFPI if the concentrations of the TFPI biological activity standard are multiplied by 0.85 prior to plotting, so that the activity plotted is equivalent to the activity of 100% pure reference ala-TFPI. When the clotting time for a particular TFPI analog preparation is compared to the standard curve, the equivalent concentration of reference ala-TFPI can be read off the curve. Alternatively, if the slope of the linear portion of the standard curve is obtained by linear regression analysis, then the slope can be corrected based on the activity of the TFPI biological activity standard relative to reference ala-TFPI. The relative biological activity of a particular TFPI analog preparation is thus equal to the ratio of reference ala-TFPI activity to the activity of the analog preparation. For example, if a particular analog requires 1.43 μg to produce the same prothrombin time activity as 1.00 μg of reference ala-TFPI, then the relative biological activity of the analog preparation is 1.00/1.43, or 0.7. For that analog preparation, the equivalent dose to a reference ala-TFPI dose is obtained by dividing the reference ala-TFPI dose by the relative biological activity of the analog preparation. For example, a 0.025 mg/kg/hr dose for reference ala-TFPI would be equivalent to 0.0357 mg/kg/hr of the preparation of the analog (*i.e.*, 0.025/0.7).

[55] The biological activity of TFPI and TFPI analogs can be determined by the prothrombin assay. Suitable prothrombin assays are described in U.S. Patent 5,888,968 and in WO 96/40784. Briefly, prothrombin time can be determined using a coagulometer (*e.g.*, Coag-A-Mate MTX II from Organon Teknika). A suitable assay buffer is 100 mM NaCl, 50 mM Tris adjusted to pH 7.5, containing 1 mg/ml bovine serum albumin. Additional reagents required are normal human plasma (*e.g.*, "Verify 1" by Organon Teknika), thromboplastin reagent (*e.g.*, "Simplastin Excel" by Organon Teknika), and TFPI standard solution (*e.g.*, 20 µg of 100% pure ala-TFPI (or equivalent thereof) per ml of assay buffer). A standard curve is obtained by analyzing the coagulation time of a series of dilutions of the TFPI standard solution, *e.g.*, to final concentrations ranging from 1 to 5 µg/ml. For the determination of clotting time, the sample, or TFPI standard, is first diluted into the assay buffer. Then normal human plasma is added. The clotting reaction is started by the addition of thromboplastin reagent. The instrument then records the clotting time. A linear TFPI standard curve is obtained from a plot of log clotting time vs. log TFPI concentration. The standard curve is adjusted based on the purity of the TFPI standard to correspond to the equivalent TFPI concentration of a 100% pure standard. For example, if the standard is a preparation of ala-TFPI which is 97% biochemically pure (*i.e.*, it contains 3% by weight of molecular species without biological activity of TFPI), then the concentration of each dilution of the standard is multiplied by 0.97 to give the actual concentration of TFPI. Thus, a TFPI standard which is 1.0 µg/ml based on the actual weight per ml of a preparation which is 97% pure will be equivalent to, and treated as, a concentration of 1.0 x 0.97, or 0.97 µg/ml.

[56] As utilized herein, the term "sepsis" means a toxic condition resulting from the spread of bacterial endotoxins from a focus of infection, as well as the spread of microorganisms or their products.

[57] As utilized herein, the term "sepsis-associated coagulation disorder" means a disorder resulting from or associated with coagulation system activation by bacterial endotoxin or by microorganisms and their products. An example of such sepsis-associated coagulation disorder is disseminated intravascular coagulation.

[58] Generally, TFPI and TFPI analogs may be useful for those diseases that occur due to the up-regulation of tissue factor and hence TF activity brought on by TNF, IL-1 or other cytokines. Low dose TFPI administration can lower the IL-6 concentrations or other cytokine concentrations in a patient. Low dose TFPI administration is useful for treating inflammation generally, including both acute and chronic inflammation. Typical inflammatory conditions that can be treated by low dose TFPI include: arthritis, septic shock, reperfusion injury, inflammatory bowel disease, acute respiratory disease (including acute respiratory distress syndrome or ARDS), trauma, and burn. In treating chronic or acute inflammation, TFPI and TFPI analogs may be administered in the same fashion and at the same doses as in the anti-sepsis method.

[59] TFPI and TFPI analogs may also be used to treat conditions resulting from the presence of bacterial endotoxins or the presence of microorganisms and their products in the circulation. Such conditions include bacteremia, peritonitis, multiple organ failure resulting from septic shock, and DIC, as well as severe pneumonia and multiple organ failure.

[60] "TFPI" and "mature, full-length TFPI" as used herein both refer to the mature polypeptide that contains 276 amino acid residues and whose sequence is shown in SEQ ID NO:1.

[61] The term "TFPI analog" refers to derivatives of the TFPI molecule, *i.e.*, molecules containing the full 276 amino acid sequence of human TFPI (SEQ ID NO:1) modified with one or more amino acid additions or substitutions (generally conservative in nature), one or more amino acid deletions, or the addition of one or more chemical moieties to one or more amino acids, so long as the modifications do not destroy TFPI biological activity. Methods for making polypeptide analogs are known in the art and are described further below. A preferred TFPI analog is N-L-alanyl-TFPI (ala-TFPI), whose amino acid sequence is shown in SEQ ID NO:2. TFPI analogs possess some measure of the activity of TFPI as determined by a bioactivity assay as described below. A preferred bioactivity assay for TFPI and analogs is the prothrombin time (PT) assay (see above).

[62] Particularly preferred TFPI analogs include substitutions that are conservative in nature, *i.e.*, those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cysteine, serine threonine, and tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 1-70 conservative or non-conservative amino acid substitutions, such as 1, 2, 3, 4, 5, 6-50, 15-25, 5-10, or any integer from 1 to 70, so long as the desired function of the molecule remains intact. One of skill in the art may readily determine regions of the molecule of interest that can be modified with a reasonable likelihood of retaining biological activity as defined herein.

[63] "Homology" refers to the percent similarity between two polynucleotide or two polypeptide moieties. Two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 50%, preferably at least about 75%, more preferably at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence homology, or any percent homology between the specified ranges, over a defined length of the molecules. As used herein, "substantially homologous" also refers to sequences showing complete identity to the specified polypeptide sequence.

[64] In general, "identity" refers to an exact amino acid-to-amino acid correspondence of two polypeptide sequences, respectively. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100.

[65] Preferably, naturally or non-naturally occurring TFPI analogs have amino acid sequences which are at least 70%, 80%, 85%, 90% or 95% or more homologous to TFPI derived from SEQ ID NO:1. More preferably, the molecules are 98% or 99% homologous. Percent homology is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, and a BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman, Adv. Appl. Math. 2:482-489 (1981).

[66] TFPI and its fragments and analogs according to the invention can be either glycosylated or non-glycosylated. Mature, full-length human TFPI is a serum glycoprotein with 276 amino acids and a molecular weight of about 38,000 Daltons. It is a natural inhibitor of tissue factor activity and thus coagulation activation. U.S. Pat. No. 5,110,730 describes tissue factor (TF), and U.S. Pat. No. 5,106,833 describes TFPI. The cloning of the TFPI cDNA is described in Wun et al., U.S. Pat. No. 4,966,852. Fragments and analogs of TFPI are described in U.S. Pat. No. 5,106,833. Ala-TFPI is a TFPI analog which is also known under the international drug name "tifacogin." Ala-TFPI includes the entire amino acid sequence of mature, full-length human TFPI plus an additional alanine residue at the amino terminus. The amino terminal alanine residue of ala-TFPI was engineered into the TFPI sequence to improve *E. coli* expression and to effect cleavage of what would otherwise be an amino terminal methionine residue. *See* U.S. Patent Nos. 4,966,852 and 5,212,091. TFPI and analogs of TFPI used in the methods of the invention can be produced from cells or tissues or produced recombinantly in either prokaryotic or eukaryotic cells.

[67] Mature, full-length TFPI is a protease inhibitor and has 3 Kunitz domains, two of which are known to interact with factors VII and Xa respectively. The function of the third domain remains unknown. TFPI is believed to function *in vivo* to limit the initiation of coagulation by forming an inert, quaternary factor $X_a$:TFPI:factor $VII_a$:tissue factor complex. *See* reviews by Rapaport, Blood 73:359-365 (1989) and Broze et al., Biochemistry 29:7539-7546 (1990). Many of the structural features of TFPI can be deduced from its homology with other well-studied protease inhibitors. TFPI is not an

enzyme, so it probably inhibits its protease target in a stoichiometric manner, *i.e.*, one of the Kunitz domains of TFPI inhibits one protease molecule. Preferably, Kunitz domains 1 and/or 2 will be present in TFPI molecules of the instant invention. The function of Kunitz domain 3 is unknown.

*Obtaining TFPI and TFPI analogs*

[68] TFPI and analogs of TFPI used in the methods of the invention can be isolated and purified from cells or tissues, chemically synthesized, or produced recombinantly in either prokaryotic or eukaryotic cells.

[69] Ala-TFPI has been expressed as a recombinant non-glycosylated protein using *E. coli* host cells. Methods have been described which yield a highly active ala-TFPI by *in vitro* refolding of the recombinant protein produced in *E. coli*. *See, e.g.,* WO 96/40784.

[70] TFPI also has been expressed as a recombinant glycosylated protein using mammalian cell hosts including mouse C127 cells as disclosed by Day et al., Blood 76:1538-1545 (1990), baby hamster kidney cells as reported by Pedersen et al., J. Biol Chem. 265:16786-16793 (1990), Chinese hamster ovary cells and human SK hepatoma cells. The C127 TFPI has been used in animal studies and shown to be effective in the inhibition of tissue factor-induced intravascular coagulation in rabbits (Day et al., *supra*), in the prevention of arterial reocclusion after thrombolysis in dogs (Haskel et al., Circulation 84:821-827 (1991)), and in reduction of mortality in an *E. coli* sepsis model in baboons (Creasey et al., J. Clin. Invest. 91:2850 (1993)).

[71] TFPI can be isolated by several methods. For example, cells that secrete TFPI include aged endothelial cells or young endothelial cells which have been treated with TNF for about 3 to 4 days, also hepatocytes or hepatoma cells. TFPI can be purified from this cell culture by conventional methods. For example, these methods include the chromatographic methods shown in Pedersen et al., 1990, J. of Biological Chemistry, 265:16786-16793, Novotny et al., 1989, J. of Biological Chemistry, 264:18832-18837, Novotny et al., 1991, Blood, 78:394-400, Wun et al., 1990, J. of Biological Chemistry, 265;16096-16101, and Broze et al., 1987, PNAS (USA), 84:1886-1890. Furthermore,

TFPI appears in the bloodstream and could be purified from blood, *see* Pedersen et al., *supra*. However, large quantities of blood would be required to obtain sufficient quantities of TFPI.

[72] TFPI and TFPI analogs may be produced recombinantly as shown in U.S. Pat. No. 4,966,852. For example, the cDNA for the desired protein can be incorporated into a plasmid for expression in prokaryotes or eukaryotes. U.S. Pat. No. 4,847,201 provides details for transforming microorganisms with specific DNA sequences and expressing them. There are many other references known to those of ordinary skill in the art which provide details on expression of proteins using microorganisms. Many of those are cited in U.S. Pat. No. 4,847,201, such as Maniatas, T., et al., 1982, Molecular Cloning, Cold Spring Harbor Press.

[73] A variety of techniques are available for transforming microorganisms and using them to express TFPI and TFPI analogs. The following are merely examples of possible approaches. TFPI DNA sequences must be isolated and connected to the appropriate control sequences. TFPI DNA sequences are shown in U.S. Pat. No. 4,966,852 and can be incorporated into a plasmid, such as pUC13 or pBR322, which are commercially available from companies such as Boehringer-Mannheim. Once the TFPI DNA is inserted into a vector, it can be cloned into a suitable host. The DNA can be amplified by techniques such as those shown in U.S. Pat. No. 4,683,202 to Mullis and U.S. Pat. No. 4,683,195 to Mullis et al. TFPI cDNA may be obtained by inducing cells, such as hepatoma cells (such as HepG2 and SKHep) to make TFPI mRNA, then identifying and isolating the mRNA and reverse transcribing it to obtain cDNA for TFPI. After the expression vector is transformed into a host such as *E. coli*, the bacteria may be fermented and the protein expressed. Bacteria are preferred prokaryotic microorganisms and *E. coli* is especially preferred. A preferred microorganism useful in the present invention is *E. coli* K-12, strain MM294 deposited with the ATCC on Feb. 14, 1984, under the provisions of the Budapest Treaty. It has accession number of 39607.

[74] It is also, of course, possible to express genes encoding polypeptides in eukaryotic host cell cultures derived from multicellular organisms. *See*, for example, Tissue Culture,

1973, Cruz and Patterson, eds., Academic Press. Useful mammalian cell lines include murine myelomas N51, VERO, HeLa cells, Chinese hamster ovary (CHO) cells, COS, C127, Hep G2, and SK Hep. TFPI and analogs can also be expressed in baculovirus-infected insect cells (*see also* U.S. Pat. Nos. 4,847,201; 5,348,886; and 4,745,051). *See also* Pedersen et al., 1990, J. of Biological Chemistry, 265:16786-16793. Expression vectors for eukaryotic cells ordinarily include promoters and control sequences compatible with mammalian cells such as, for example, the commonly used early and later promoters from Simian Virus 40 (SV40) (Fiers, et al., 1978, Nature, 273:113), or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters. General aspects of mammalian cell host system transformations have been described by Axel, U.S. Pat. No. 4,399,216, issued Aug. 16, 1983. It now appears also that "enhancer" regions are important in optimizing expression; these are, generally, sequences found upstream of the promoter region. Origins of replication may be obtained, if needed, from viral sources. However, integration into the chromosome is a common mechanism for DNA replication in eukaryotes. Plant cells are also now available as hosts, and control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequences (Depicker, A., et al., 1982, J. Mol. Appl. Gen., 1:561) are available. Methods and vectors for transformation of plant cells have been disclosed in PCT Publication No. WO 85/04899, published Nov. 7, 1985.

[75] Methods which can be used for purification of TFPI and TFPI analogs expressed in mammalian cells include sequential application of heparin-Sepharose, MonoQ, MonoS, and reverse phase HPLC chromatography. See Pedersen et al., supra; Novotny et al., 1989, J. Biol. Chem. 264:18832-18837; Novotny et al., 1991, Blood, 78:394-400; Wun et al., 1990, J. Biol. Chem. 265:16096-16101; Broze et al., 1987, PNAS (USA), 84:1886-1890; U.S. Pat. No. 5,106,833; and U.S. Patent No. 5,466,783. These references describe various methods for purifying mammalian produced TFPI.

[76] Additionally, TFPI and TFPI analogs may be produced in bacteria or yeast and subsequently purified. Generally, the procedures shown in U.S. Pat. Nos. 5,212,091;

6,063,764; and 6,103,500 or WO 96/40784 can be employed. Ala-TFPI and other TFPI analogs can be purified, solubilized, and refolded according WO 96/40784 and Gustafson et al., Prot. Express. Pur. 5:233 (1994), which are incorporated herein by reference. For example, when prepared according Example 9 of WO 96/40784, preparations of ala-TFPI may be obtained that contain from about 85% to 90% of the total protein by weight as mature, properly-folded, biologically active ala-TFPI, about 10% to 15% of which has one or more oxidized methionine residues. These oxidized forms have biological activity that is equivalent to the biological activity of underivatized ala-TFPI, as determined by prothrombin assay, and are expected to be active in the invention disclosed herein. The remaining material comprises various modified forms of ala-TFPI, including dimerized, aggregated, and acetylated forms.

[77] TFPI and TFPI analogs may have a significant number of cysteine residues and the procedure shown in U.S. Pat. No. 4,929,700 is relevant to TFPI refolding. TFPI and analogs may be purified from the buffer solution by various chromatographic methods, such as those mentioned above. Additionally, the methods shown in U.S. Pat. No. 4,929,700 may be employed. Any method may be employed to purify TFPI and TFPI analogs which results in a degree of purity and a level of activity suitable for administration to humans.

[78] A TFPI or TFPI variant can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of TFPI or TFPI variants can be separately synthesized and combined using chemical methods to produce a full-length molecule.

*Formulations*

[79] In accordance with this invention, formulations of TFPI and TFPI analogs are preferably administered by intravenous infusions. Essentially continuous intravenous infusion is preferred. Methods to accomplish this administration are known to those of ordinary skill in the art. Infusion can be performed via a central line (preferred) or a peripheral line. While large fluctuations in the dose rate are to be avoided, short-term deviations from the dose rates of the invention are acceptable provided the resulting plasma level of administered TFPI is within 20% of that expected from a continuous infusion at a constant dose rate according to the preferred embodiments of invention.

[80] Before administration to patients, formulants may be added to TFPI and TFPI analogs. A liquid formulation is preferred. TFPI and TFPI analogs may be formulated at different concentrations, using different formulants, and at any physiologically suitable pH compatible with the route of administration, solubility, and stability of the TFPI protein. A preferred formulation for intravenous infusion includes ala-TFPI at up to about 0.6 mg/ml, arginine hydrochloride at up to 300 mM, and sodium citrate buffer at pH 5.0-6.0. Certain solutes such as arginine, NaCl, sucrose, and mannitol serve to solubilize and/or stabilize ala-TFPI. *See* WO 96/40784. An especially preferred formulation for intravenous infusion contains about 0.15 mg/ml reference ala-TFPI, 300 mM arginine hydrochloride, and 20 mM sodium citrate at pH 5.5. TFPI and TFPI analogs also can be formulated at concentrations up to about 0.15 mg/ml in 150 mM NaCl and 20 mM sodium phosphate or another buffer at pH 5.5-7.2, optionally with 0.005% or 0.01% (w/v) polysorbate 80 (Tween 80). Other formulations contain up to about 0.5 mg/ml TFPI, or TFPI analog in 10 mM sodium acetate at pH 5.5 containing either 150 mM NaCl, 8% (w/v) sucrose, or 4.5% (w/v) mannitol. TFPI and TFPI analogs can also be formulated at higher concentrations up to several mg/ml using high salt. For example, one formulation contains up to about 6.7 mg/ml ala-TFPI in 500 mM NaCl and 20 mM sodium phosphate at pH 7.0.

[81] Further examples of formulants for TFPI and TFPI analogs include oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking

agents. Preferably carbohydrates include sugar or sugar alcohols such as mono, di, or polysaccharides, or water soluble glucans. The saccharides or glucans can include fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. Sucrose is most preferred. Sugar alcohol is defined as a $C_4$ to $C_8$ hydrocarbon having an -OH group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. Mannitol is most preferred. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. Preferably, the sugar or sugar alcohol concentration is between 1.0 w/v % and 7.0 w/v %, more preferable between 2.0 and 6.0 w/v %. Preferably amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. It is also preferred to use a buffer in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Most any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are preferred. Preferably, the concentration of the buffer is from 0.01 to 0.3 molar. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

[82] Additionally, TFPI and TFPI analogs can be chemically modified, for example by covalent conjugation to a polymer to increase its circulating half-life. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Pat. Nos. 4,766,106, 4,179,337, 4,495,285, and 4,609,546. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O--CH_2--CH_2)_n--O--R$ where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a

preferred average molecular weight between 1000 and 40,000, more preferably between 2000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/TFPI of the present invention.

[83] Water soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), etc. POG is preferred. One reason is because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in Knauf et al., 1988, J. Bio. Chem. 263:15064-15070, and a discussion of POG-protein conjugates is found in U.S. Pat. No. 4,766,106, both of which are hereby incorporated by reference in their entireties.

[84] While TFPI and TFPI analogs can be administered as the sole active anticoagulation pharmaceutical agent, it can also be used in combination with one or more additional therapeutic agents. Such additional therapeutic agents include antibodies useful for treating sepsis, such as, for example, anti-endotoxin, monoclonal antibodies (*e.g.*, endotoxin-binding Mabs) and anti-TNF products such as an anti-TNF murine Mab. TFPI and TFPI analogs can also be combined with interleukin-1 receptor antagonists, bactericidal/permeability increasing (BPI) protein, immunostimulant, compounds having anti-inflammatory activity such as PAF antagonists (*e.g.*, Pafase, platelet-activating factor acetylhydrolase), and cell adhesion blockers (*e.g.*, antiplatelet agents such as GPIIb/IIIa inhibitors). When administered as a combination, the therapeutic agents can be formulated as separate compositions which are given at the same time or different times, or the therapeutic agents can be given as a single composition.

[85] TFPI and TFPI analogs may be given in combination with other agents which would be effective to treat sepsis. For example, the following may be administered in combination with TFPI and TFPI analogs: antibiotics that can treat the underlying bacterial infection; monoclonal antibodies that are directed against bacterial cell wall components; receptors that can complex with cytokines that are involved in the sepsis pathway; low molecular weight heparin if required for prophylactic therapy unrelated to sepsis, and generally any agent or protein that can interact with cytokines or complement proteins in the sepsis pathway to reduce their effects and to attenuate sepsis or septic shock.

[86] Antibiotics that are useful in the present invention include those in the general category of: beta-lactam rings (penicillin), amino sugars in glycosidic linkage (aminoglycosides), macrocyclic lactone rings (macrolides), polycyclic derivatives of napthacenecarboxanide (tetracyclines), nitrobenzene derivatives of dichloroacetic acid, peptides (bacitracin, gramicidin, and polymyxin), large rings with a conjugated double bond system (polyenes), sulfa drugs derived from sulfanilamide (sulfonamides), 5-nitro-2-furanyl groups (nitrofurans), quinolone carboxylic acids (nalidixic acid), and many others. Other antibiotics and more versions of the above specific antibiotics may be found in Encyclopedia of Chemical Technology, 3rd Edition, Kirk-Othymer (ed.), Vol. 2, pages 782-1036 (1978) and Vol. 3, pages 1-78, Zinsser, MicroBiology, 17th Edition W. Joldik et al. (Eds.) pages 235-277 (1980), or Dorland's Illustrated Medical Dictionary, 27th Edition, W. B. Saunders Company (1988).

[87] Other agents which may be combined with TFPI and TFPI analogs include endotoxin antagonists such as E5531 (a Lipid A analog, *see* Asai et al., Biol. Pharm. Bull. 22:432 (1999)); TF analogs with anticoagulant activity (*see, e.g.,* Kelley et al., Blood 89:3219 (1997) and Lee & Kelley, J. Biol. Chem. 273:4149 (1998)); monoclonal antibodies directed to cytokines involved in the sepsis pathway, such as those monoclonal antibodies directed to IL-6 or M-CSF, *see* U.S. Ser. No. 07/451,218, filed Dec. 15, 1989 to Creasey et al. and monoclonal antibodies directed to TNF, *see* Cerami et al., U.S. Pat. No. 4,603,106; inhibitors of protein that cleave the mature TNF prohormone from the cell in which it was produced, *see* U.S. Ser. No. 07/395,253, filed Aug. 16, 1989, to Kriegler et

al.; antagonists of IL-1, such as shown in U.S. Ser. No. 07/517,276, filed May 1, 1990 to Haskili et al.; inhibitors of IL-6 cytokine expression such as inhibin, as shown in U.S. Patent 5,942,220, issued August 24, 1999 to Warren et al.; and receptor based inhibitors of various cytokines such as IL-1. Antibodies to complement or protein inhibitors of complement, such as $CR_1$, DAF, and MCP may also be employed.

[88] After the liquid pharmaceutical composition is prepared, it can be lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) which may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects by continuous intravenous infusion.

[89] As stated above, TFPI and TFPI analogs are useful to therapeutically or prophylactically treat human patients with sepsis or septic shock, with or without DIC. Generally, people having sepsis are characterized by high fever (>38.5° C) or hypothermia (<35.5° C), low blood pressure, tachypnea (>20 breaths/minute), tachycardia (>100 beats/minute), leukocytosis (>15,000 cells/$mm^3$) and thrombocytopenia (<100,000 platelets/$mm^3$) in association with bacteremia. TFPI and TFPI analogs should be administered as soon as a patient is suspected of being septic, *i.e.*, presenting with a greater than or equal to 20% drop in fibrinogen or appearance of fibrin split products or other biochemical changes, a rise in the patient's temperature, and the diagnosis of leukopenia, thrombocytopenia, and hypotension associated with sepsis, or presenting with Systemic Inflammatory Response, including a drop in blood pressure (hypotension), an increase or decrease in temperature, an increase in respiratory rate, leukopenia, thrombocytopenia, etc., and suspicion of an infection that warrants systemic anti-infective therapy. TFPI can also be administered to patients who are at risk for sepsis, for example, from a gunshot wound or from a surgical incision. Patients who are at risk for sepsis also include patients admitted to a hospital emergency room or intensive care unit and who have or are suspected of having a focus of infection or systemic inflammatory response syndrome (SIRS).

[90] The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

## EXAMPLES

**Example 1.** Co-administration of Ala-TFPI and Heparin to Healthy Human Subjects

[91] Non-glycosylated ala-TFPI expressed in *E. coli* was administered together with heparin to healthy volunteers. Heparin was administered by IV infusion or subcutaneous injection at doses of 300 or 5000 U, respectively, after 6 hr of an 11 hr ala-TFPI infusion (about 0.5 mg/kg/hr). Transient increases in ala-TFPI levels were observed following heparin administration by either route, potentially due to displacement of ala-TFPI from endothelial binding sites. PT and activated partial thromboplastin time (aPTT) values during this time were variable but generally rose slightly and then fell in parallel with changes in ala-TFPI concentrations. These doses of heparin were safe and well-tolerated when used in combination with ala-TFPI.

**Example 2.** Treatment of Sepsis in Humans with High Doses of Ala-TFPI

[92] Fourteen subjects were recruited as part of a Phase II clinical study of non-glycosylated ala-TFPI therapy in severe sepsis. The study was a Phase II, multi-center, double-blind, randomized, placebo-controlled, two-step dose-escalation, safety, and tolerability study in subjects with severe sepsis. The primary objective was to evaluate the safety profile of ala-TFPI. The secondary objectives were to evaluate the pharmacokinetics, cytokine and coagulation markers, multi-organ failure scores, and 15 and 28 day survival. Five subjects were randomized to placebo, five to an ala-TFPI dose rate of about 0.33 mg/kg/hr, and four to a dose rate of about 0.66 mg/kg/hr (both doses by continuous intravenous infusion). The study was terminated prematurely due to higher than anticipated anticoagulation (elevated INR) and bleeding.

[93] An adverse event (AE) was defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, at any dose, which

did not necessarily have a causal relationship with the treatment. An AE could, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of the product, whether or not considered related to the medicinal product. A serious adverse event (SAE) was defined as any untoward medical occurrence that, at any dose, resulted in death, was life threatening (*i.e.*, the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred), required or prolonged a subject's hospitalization, resulted in persistent or significant disability/incapacity (*i.e.*, the event caused a substantial disruption of a person's ability to conduct normal life functions), was a congenital anomaly/birth defect, or was an important medical event that, based upon appropriate medical judgment, may have jeopardized the subject and may have required medical or surgical intervention to prevent one of the other outcomes defined as a serious adverse event.

[94] Seven of nine subjects receiving ala-TFPI experienced bleeding compared to one of five subjects receiving placebo. In addition, three ala-TFPI recipients had a markedly elevated INR while on active infusion. In two subjects, the INR rapidly declined after the infusion was discontinued. In one subject, the INR returned to baseline although the infusion was not interrupted. No bleeding events were reported while the INR levels were elevated. The most common SAEs other than bleeding were due to sepsis and its related complications.

[95] One of five subjects who received placebo, five of five who received ala-TFPI at about 0.33 mg/kg/hr, and two of four who received ala-TFPI at about 0.66 mg/kg/hr experienced an adverse event involving bleeding. Two subjects out of five (40%) in the placebo group, four subjects out of five (80%) in the ala-TFPI at 0.33 mg group, and all four subjects in the ala-TFPI at 0.66 mg group experienced at least one SAE during the study.

[96] A summary of SAEs is presented in Table 1. Note that subjects can experience more than one SAE.

**Table 1.** Summary of SAEs

| Body System/COSTART term | Placebo (n=5) | 0.33 mg/kg/hr ala-TFPI (n=5) | 0.66 mg/kg/hr ala-TFPI (n=4) |
|---|---|---|---|
| **Body as a whole** | | | |
| Sepsis | 0 | 0 | 1 |
| Shock | 0 | 1 | 2 |
| **Cardiovascular system** | | | |
| Atrial fibrillation | 0 | 1 | 0 |
| Hemorrhage | 0 | 1 | 1 |
| Hypotension | 1 | 1 | 1 |
| **Digestive system** | | | |
| Gastrointestinal hemorrhage | 0 | 2 | 0 |
| **Hemic and lymphatic system** | | | |
| Prothrombin increased | 0 | 1 | 1 |
| **Nervous system** | | | |
| Brain edema | 0 | 0 | 1 |
| Brain stem disorder | 0 | 0 | 1 |
| **Respiratory system** | | | |
| Atelectasis | 1 | 0 | 0 |
| Pneumothorax | 0 | 1 | 0 |
| Respiratory disorder | 0 | 0 | 1 |
| **Urogenital system** | | | |
| Acute kidney failure | 0 | 0 | 1 |

[97] A total of four SAEs involving bleeding and two SAEs related to unexpected prolongation of PT occurred in the ala-TFPI groups. The other SAEs were due to complications of severe sepsis.

**Example 3.** Effectiveness of Low Doses of TFPI in a Rabbit Peritonitis Model

[98] Studies in rabbits indicated that lower doses of ala-TFPI than those described in Example 2 could be efficacious. Five different groups receiving low doses of non-glycosylated ala-TFPI were evaluated in the rabbit *E. coli* peritonitis model. Because an ala-TFPI dose of about 0.5 mg/kg bolus and about 5 mg/kg/min infusion for 24 hr (human equivalent, 0.8 mg/kg/hr) rescued 58% of the rabbits (Table 2), it served as the positive control. Significant differences in the percent survival between ala-TFPI dose groups and placebo were seen for all doses except the lowest dose (Table 2), which showed the same percent survival as the placebo-treated controls in these studies (20%).

[99] Rabbits were inoculated intraperitoneally with a suspension containing hemoglobin (40 μg/ml), porcine mucin (150 μg/ml), and viable *E. coli* strain O18:K1+ ($1.0 \pm 0.5 \times 10^5$ CFU/kg), a clinical isolate that causes ≥20% of all cases of *E. coli* sepsis in humans. Treatment with gentamicin (5 mg/kg every 12 hr for 5 doses) was initiated 4 hr after induction of peritonitis. After 4 hr, clinical symptoms of fever, chills, and a slight drop in blood pressure typically were present. At that time point, rabbits were randomized to receive a 24-hr infusion of placebo or one of six doses of ala-TFPI, as shown in Table 2, consisting of a bolus dose followed by continuous intravenous infusion. Rabbits remaining alive after seven days were considered survivors. The cause of death in this model is respiratory and multiple organ failure. At necropsy, the lungs of nonsurvivors were grossly edematous, and the airways were filled with pink, frothy fluid. The kidneys were hemorrhagic and congested. The liver typically contained abscesses that grew out *E. coli.* Abscesses were present throughout the peritoneal cavity. In contrast, 7-day survivors showed evidence of minimal pulmonary congestion. Small scattered abscesses often were present in the peritoneal cavity, but other solid organs (adrenals, liver, and spleen) were grossly normal.

**Table 2.** Efficacy of ala-TFPI at lower doses in a rabbit focus-of-infection model of sepsis.

| ala-TFPI dose | | % Survival* | Circulating ala-TFPI level (μg/ml) | Human equivalent dose (mg/kg/hr) |
|---|---|---|---|---|
| Bolus (mg/kg) | Continuous infusion (μg/kg/min) | | | |
| 0.5 | 5 | 58 (*P*=.004) | 0.8 | 0.8 |
| 0.05 | 0.5 | 36 (*P*=.004) | 0.4 | 0.2 |
| 0.05 | 0.05 | 66 (*P*=.05) | 0.14 | 0.07 |
| 0.0001 | 0.001 | 73 (*P*=.007) | <0.002 | <0.002 |
| 0.00001 | 0.0001 | 41 *(P=0.20)* | — | — |
| 0.000001 | 0.00001 | 20 | — | — |
| Control | | 20 | — | — |

**Example 4.** Safety and Efficacy of Low Doses of Ala-TFPI in Treatment of Septic Human Patients

[100] This example describes the clinical use of low dose administration of ala-TFPI to treat patients with severe sepsis.

[101] A Phase II single-blinded dose escalation study in subjects with severe sepsis was performed to evaluate dose rates of 0.025 and 0.05 mg/kg/hr non-glycosylated ala-TFPI produced in *E. coli* versus placebo. Ala-TFPI was administered by continuous intravenous infusion (CIV) infusion for up to 96 hours in 210 subjects (placebo: n=69; about 0.025 mg/kg/hr ala-TFPI: n=80; about 0.05 mg/kg/hr ala-TFPI: n=61). Subjects were enrolled in blocks of 30 starting at the 0.025 mg/kg/hr ala-TFPI dose. Dosing stage 1 included subjects in the 0.025 mg/kg/hr ala-TFPI and placebo groups. Dosing stage 2 included subjects in the 0.05 mg/kg/hr ala-TFPI and placebo groups. The study indicated that ala-TFPI at a dose rate of about 0.025 mg/kg/hr was safe and was associated with trends towards reduction in 28-day all cause mortality and improvement in some multiple

organ dysfunction (MOD) scores relative to placebo. MOD scores were assigned according to the scoring criteria listed in Table 3.

[102] Patients were enrolled into the study within 24 hours of onset of severe sepsis. During the entire period of evaluation, patients received full intensive care management, including fluid resuscitation, vasopressors, ventilatory support, parenteral antimicrobial agents, and appropriate surgical management.

[103] The protocol initially called for different groups of patients to receive ala-TFPI dose levels ranging from 0.025 mg/kg/hr to 0.1 mg/kg/hr, proceeding successively from lower to higher dose levels. Subjects were enrolled sequentially in blocks of 30 starting at the 0.025 mg/kg/hr ala-TFPI dose level. Two hundred ten subjects were randomized and infused in a 1:2 ratio (placebo to ala-TFPI) into dosing stage 1 (S1) (placebo [n=39] and about 0.025 mg/kg/hr ala-TFPI [n=80]) or dosing stage two (S2) (placebo [n=30] and about 0.05 mg/kg/hr ala-TFPI [n=61]) and received a continuous intravenous infusion (CIV) of placebo or ala-TFPI for up to 4 days.

[104] Eligible subjects for this study were patients of either sex and age ≥ 18 years, who gave informed consent, and who fulfilled all of the following criteria: (1) clinical evidence of infection, with a clearly verifiable focus of infection; (2) signs of systemic inflammatory response syndrome (SIRS) (signs of SIRS consisted of fever (core, rectal, axillary or tympanic temperature ≥ 38°C) or hypothermia (core or rectal temperature of ≤ 36°C), heart rate > 90 per minute, respiratory rate > 20 per min or $PaCO_2$ (alveolar partial pressure of $CO_2$) < 32 mm Hg, or if the subject was on a ventilator, white blood cell count > 12,000/$mm^3$ , < 4,000/$mm^3$ , or > 10% immature band forms); (3) at least one of sign of organ dysfunction/hypoperfusion (OD) as evidenced by pulmonary dysfunction ($PaO_2/FiO_2$ ratio (ratio of alveolar partial pressure of $O_2$ to fraction of inhaled $O_2$) < 250 (or < 200 in the presence of pneumonia or other localized lung disease), metabolic acidosis (pH ≤ 7.30 or base deficit ≥ 5.0 mEq/L (≥ 5.0 mmol/L) thought to be due to lactic acidosis or increased plasma lactate concentration, oliguria (urine output ≤ 0.5 mL/kg/hr for a minimum of 2 consecutive hours in the presence of adequate fluid

resuscitation (not valid as organ dysfunction if subject was on chronic dialysis)), unexplained thrombocytopenia (platelet count ≤ 100,000 cell/mm$^3$) not presumed to be drug-induced—this criterion could not be used in subjects who had received therapies likely to have caused thrombocytopenia, or hypotension (vasopressors required at therapeutic doses (*i.e.*, dopamine > 5 μg/kg/min or any dose of epinephrine, norepinephrine, or phenylephrine to maintain systolic blood pressure > 90 mm Hg for at least 2 consecutive hours).

**[105]** The main exclusion criteria were: (1) refractory hypotension within 2 hours of anticipated drug dosing, (2) known or suspected endocarditis, (3) uncontrolled hemorrhage; INR > 3, (4) major surgery ≤ 12 hours prior to study drug infusion, (5) history of intracranial bleeding within 6 months or closed head trauma or stroke within 1 month or other neurological condition with increased bleeding risk, (6) platelet count ≤ 20,000/mm$^3$, (7) pregnancy, (8) weight > 150 kg, (9) significant liver disease (Child Pugh grade C) and/or known or suspected esophageal varices, (10) confirmed, clinically-evident pancreatitis, (11) cardiopulmonary arrest within 72 hours prior to study entry, (12) evidence of acute or chronic transplant rejection; second or third degree burns > 10% of body surface area, (13) systemic treatment with anticoagulants, anti-platelet drugs or thrombolytics or tissue plasminogen activator, (14) daily heparin doses > 20,000 International Units, (15) receipt of an investigational new drug or biologic within 30 days of study enrollment, or (16) refusal of mechanical ventilation, dialysis or hemofiltration, cardioversion or any required drug/fluid therapy at time of consent.

**[106]** Due to the sequential enrollment into the two dose stages, each active dose group is compared with its own placebo group. In addition, data are displayed comparing all placebo versus all ala-TFPI subjects. The 0.1 mg/kg/hr dose was eliminated from the study due to serious adverse events observed at the 0.050 mg/kg/hr dose.

**[107]** The study drug, ala-TFPI, is recombinant non-glycosylated ala-TFPI expressed in *E coli* and purified by standard chromatography techniques. The study drug was supplied in vials containing a volume of 100 ml of sterile ala-TFPI solution at a concentration of 0.3

mg/ml and was formulated in an isotonic buffer. The placebo is composed of the diluent buffer and was packaged and stored under the same conditions as the study drug. Ala-TFPI was infused at the predefined dosage levels at a constant rate over the 4 day period. Drug infusion was conducted through a venous catheter (central or peripheral) dedicated for ala-TFPI or placebo.

[108] Patients were followed for 28 days or until death. Multiple organ dysfunction scores (MODS) and concomitant medications were collected through day 8. Acute physiology and chronic health evaluation (APACHE II) score and hospital laboratory INR were collected through day 4. Routine safety laboratory parameters (total bilirubin, creatinine, transaminases, serum lactic acid (baseline only) complete blood count (CBC) with differential and platelets) were collected through day 6. Specialized tests, such as research laboratory parameters (thrombin-anti-thrombin complexes (TATc), and IL-6) and pharmacokinetic samples, were collected through days 4 and 5, respectively. If clinically indicated, electrocardiograms (ECG) and chest x-rays were collected though day 6. Patients were monitored through day 28 for adverse events (AEs) and all causes of mortality.

[109] The INR was measured with a bedside monitor (CoaguCheck Plus, Roche Diagnostics, Nutley, New Jersey) and periodically by hospital laboratory. Ala-TFPI kinetics and activity were measured by the effect on INR response. Another method that was used is the determination of thrombin-antithrombin complexes (TATc). TATc samples were obtained at pre-infusion (-2 hours to time 0) and on days 2, 3 and 4. The hospital INR was obtained within 6 hours of screening, and on days 1 (4 hours), 2, 3, and 4; bedside INR was obtained at pre-infusion, 1 hour, 2 hours, 3 hours, 4 hours and then every 6 hours ± 2 hours during dosing, just prior to dose termination, 4 and 8 hours post-dose termination. Dosing adjustment and/or dose discontinuation was guided by INR elevations. Unscheduled samples (dose reductions) were collected just before and 4 hours post-dose reduction.

[110] Recombinant ala-TFPI plasma concentrations were collected pre-infusion (-2 hours to time 0), on days 1 (4 and 8 hours), 2, and 3, as well as at termination of dosing.

Pharmacokinetic samples were measured using a validated electrochemiluminescent immunoassay using monoclonal and polyclonal antibodies to TFPI. The monoclonal antibody was specific for the first Kunitz domain of TFPI. As a result the assay measured endogenous TFPI as well as the recombinant form (ala-TFPI). The assay standards and quality controls were diluted in rabbit plasma. Because rabbit plasma does not contain immunologically cross-reactive TFPI, the assay can measure endogenous TFPI in human plasma. The lower limit of quantitation was 5 ng/ml.

[111] During patient enrollment in the first two dose stages, a trend was seen towards an increase in SAEs involving bleeding in the 0.05 mg/kg/hr ala-TFPI group compared to the 0.025 mg/kg/hr ala-TFPI and placebo groups. This obviated the need to perform the planned studies with the 0.1 mg/kg/hr dose.

[112] A trend towards reduction in 28-day all cause mortality was noted in the 0.025 mg/kg/hr ala-TFPI dose group compared to placebo. Logistic regression modeling indicated the presence of a substantial treatment by baseline laboratory INR interaction effect, indicating that higher baseline INR is associated with a more pronounced ala-TFPI effect.

**Table 3.** Overall Mortality.

| | Dosing Stage 1 | | Dosing Stage 2 | | All | |
|---|---|---|---|---|---|---|
| | **Placebo n=39 [N (%)]** | **0.025 mg/kg/hr ala-TFPI n=80 [N (%)]** | **Placebo n=30 [N (%)]** | **0.05 mg/kg/hr ala-TFPI n=61 [N (%)]** | **Placebo n=69 [N (%)]** | **ala-TFPI n=141 [N (%)]** |
| 28 day all cause mortality rates: | 16 (41%) | 22 (28%) | 10 (33%) | 21 (34%) | 26 (38%) | 43 (30%) |

**Table 4.** Mortality by Baseline Lab INR [subject N in ( )].

| Subgroup | All Placebo n=69 | 0.025 mg/kg/hr ala-TFPI n=80 | 0.05 mg/kg/hr ala-TFPI n=61 |
|---|---|---|---|
| 28 day all cause mortality rates: | | | |
| All subjects | 38% (26/69) | 28% (22/80) | 34% (21/61) |
| Baseline Lab INR ≥ 1.2 | 42% (20/48) | 27% (15/56) | 40% (17/42) |
| Baseline Lab INR < 1.2 | 29% (6/21) | 29% (7/24) | 21% (4/19) |

**[113]** Multiple organ dysfunction score (MODS) was determined by using a modified Sepsis-related Organ Failure Assessment (SOFA) score (*see* Table 5). The following SOFA modifications were made. The coagulation score was modified (*see* Table 8 footnote 3) to account for platelet infusion. The renal score was modified (*see* Table 8 footnote 4) to account for dialysis. A composite intensive care unit (ICU) score was added. The ICU score combines pulmonary, cardiovascular, and coagulation scores (platelet count specifically).

**Table 5.** Scoring of Organ Dysfunction: MODS[1]

| Organ System | Lab Test/ Clinical Evaluation | Code List |
|---|---|---|
| Cardiovascular System | **Mean Arterial Pressure (mmHg)**<br>Vasopressor dose [2] | 5 = MAP ≥ 70<br>4 = MAP < 70<br>3 = dopamine ≤ 5 µg/kg/min<br>2 = dopamine > 5 µg/kg/min **OR** epinephrine/norepinephrine < 0.1 µg/kg/min **OR** any dose of phenylephrine<br>1 = dopamine > 15 µg/kg/min **OR** epinephrine/norepinephrine ≥ 0.1 µg/kg/min |
| Pulmonary System | $PaO_2$ / $FiO_2$<br>mmHg (kPa) | 5 = ≥ 400 (≥53.2)<br>4 = 300 - 399 (39.9-53.1)<br>3 = 200 - 299 (26.6-39.8)<br>2 = 100 - 199 (with respiratory support) (13.3-26.5)<br>1 = < 100 (with respiratory support) (<13.3) |
| Central Nervous System | Glasgow Coma Score | 5 = 15<br>4 = 13 - 14<br>3 = 10 - 12<br>2 = 6 - 9<br>1 = ≤ 5 |
| Coagulation System | Platelets, x $10^3/mm^3$ | 5 = ≥ 150<br>4 = 100 - 149<br>3 = 50 - 99<br>2 = 20 - 49<br>1 = < 20 |
| Renal System | Serum Creatinine,<br>mg/dl (µmol/L) | 5 = < 1.2 (<110)<br>4 = 1.2 - 1.9 (110 – 170)<br>3 = 2.0 - 3.4 (171 – 299)<br>2 = 3.5 - 4.9 ( 300 – 440) or urine output < 500 mL/24 hrs<br>1 = ≥ 5.0 (≥ 441) or urine output< 200 mL/24 hrs |
| Hepatic System | Total Bilirubin,<br>mg/dl (µmol/L) | 5 = < 1.2 (<20)<br>4 = 1.2 - 1.9 (20 – 32)<br>3 = 2.0 - 5.9 (33 – 101)<br>2 = 6.0-11.9 (102 – 204)<br>1 = ≥ 12 (≥205) |

[1] *Modified from: Vincent JL, Moreno R, et al., Intensive Care Medicine, 1996: 22:7; 707-10.*
[2] Catecholamines given for at least 1 hr.

MISSING AT THE TIME OF PUBLICATION

[114] Positive trends were observed in some organ dysfunction scores among ala-TFPI recipients of either dose compared to placebo as indicated by the higher % change from baseline. These trends were most pronounced for the pulmonary and the ICU scores.

**Table 6.** Organ Dysfunction Scores: Mean % Change from Baseline[1]

| Measure of Activity | All Placebo n=69 | 0.025 mg/kg/hr ala-TFPI n=80 | 0.05 mg/kg/hr ala-TFPI n=61 | All ala-TFPI n= 141 | p-values[2] |
|---|---|---|---|---|---|
| Pulmonary | 14% | 42% | 19% | 32% | 0.056 |
| Cardiovascular | 55% | 76% | 79% | 77% | 0.22 |
| Coagulation (platelets) | 7% | 12% | 10% | 11% | 0.39 |
| Modified Coagulation[3] | 19% | 17% | 10% | 14% | 0.59 |
| Hepatic | -3% | -3% | -5% | -4% | 0.85 |
| Renal | 16% | 9% | 8% | 9% | 0.26 |
| Modified Renal[4] | -1% | 5% | 6% | 5% | 0.36 |
| CNS | 16% | 17% | 25% | 20% | 0.67 |
| ICU Score[5] | 12% | 24% | 19% | 22% | 0.053 |
| Modified ICU Score[6] | 15% | 26% | 19% | 23% | 0.16 |
| Total MODS | 5% | 10% | 8% | 10% | 0.16 |
| Modified total MODS[7] | 4% | 10% | 7% | 9% | 0.15 |

[1] For definitions of organ dysfunctions, *see* Table 7.
[2] P-values are for all ala-TFPI subjects versus placebo.
[3] If a subject received platelets, the modified coagulation score was set to 1.
[4] If a subject received dialysis, the modified renal score was set to 1.
[5] Sum of cardiovascular, pulmonary and coagulation scores.
[6] Sum of cardiovascular, pulmonary and modified coagulation (*see* footnote 3) scores.
[7] Sum of modified renal (*see* footnote 4) and modified coagulation (*see* footnote 3) scores.

[115] Administration of ala-TFPI was interrupted if the subject's INR increased during treatment by more than 20% over the subject's baseline INR, or if the subject's INR was 2.5 or higher. The frequency of dosing interruptions due to elevated INR was low in each

group. These data suggest that INR elevations occur in severe sepsis subjects. A slight increase in dose interruptions in the ala-TFPI treated subjects as compared to placebo was noted.

**Table 7.** Subjects with INR Increases Requiring Dose Interruptions

| | Dosing Stage 1 | | Dosing Stage 2 | | All | |
|---|---|---|---|---|---|---|
| Measure of Activity | Placebo n=39 [N (%)] | 0.025 mg/kg/hr ala-TFPI n=80 [N (%)] | Placebo n=30 [N (%)] | 0.05 mg/kg/hr ala-TFPI n=61 [N (%)] | Placebo n=69 [N (%)] | ala-TFPI n=141 [N (%)] |
| Dose Interruption due to elevated INR | 5 (13%) | 11 (14%) | 1 (3%) | 8 (13%) | 6 (9%) | 19 (13%) |

[116] There were no major differences in the incidence of AEs, AEs involving bleeding, and SAEs across all body systems in the ala-TFPI groups versus placebo. The overall incidence of SAEs involving bleeding was low. There was a slight increase in the incidence of SAEs involving bleeding in the 0.05 mg/kg/hr ala-TFPI group compared to the groups receiving 0.025 mg/kg/hr ala-TFPI and placebo. The incidence of SAEs involving bleeding was also analyzed by baseline INR (≥1.2 ("elevated INR subjects") versus <1.2 ("non-elevated INR subjects")) as displayed in Table 9.

**Table 8.** Subjects with Adverse Events (AEs) and Serious Adverse Events (SAEs)

| | Dosing Stage 1 | | Dosing Stage 2 | | All | |
|---|---|---|---|---|---|---|
| **Subjects with:** | **Placebo n=39 [N (%)]** | **0.025 mg/kg/hr Ala-TFPI n=80 [N (%)]** | **Placebo n=30 [N (%)]** | **0.05 mg/kg/hr Ala-TFPI n=61 [N (%)]** | **Placebo n=69 [N (%)]** | **Ala-TFPI n=141 [N (%)]** |
| **Any AEs** | 34 (87%) | 70 (88%) | 27 (90%) | 57 (93%) | 61 (88%) | 127 (90%) |
| **Any AEs involving bleeding** | 9 (23%) | 16 (20%) | 10 (33%) | 17 (28%) | 19 (28%) | 33 (23%) |
| **Any SAEs** | 25 (64%) | 46 (58%) | 16 (53%) | 37 (61%) | 41 (59%) | 83 (59%) |
| **Any SAEs involving bleeding** | 2 (5%) | 6 (8%) | 2 (7%) | 7 (11%) | 4 (6%) | 13 (9%) |

**Table 9.** SAEs Involving Bleeding by Baseline INR

| **INR** | **All Placebo n=69 [N (%)]** | **0.025 mg/kg/hr ala-TFPI n=80 [N (%)]** | **0.05 mg/kg/hr ala-TFPI n=61 [N (%)]** |
|---|---|---|---|
| ≥ 1.2 | 3/48 (6%) | 4/56 (7%) | 7/42 (17%) |
| < 1.2 | 1/21 (5%) | 2/24 (8%) | 0/19 (0%) |

[117] There was an increase in the incidence of SAEs involving bleeding in the "elevated INR" subjects receiving about 0.05 mg/kg/hr ala-TFPI compared to about 0.025 mg/kg/hr ala-TFPI and placebo. In the cohort of "non-elevated INR" subjects the incidence of SAEs involving bleeding was low.

**Table 10.** Subject listing of bleeding adverse events

| Treatment group, site-subject number | Adverse event-bleeding (verbatim term) | Severity | Drug related | Outcome | 28-day survival status |
|---|---|---|---|---|---|
| **Placebo** | | | | | |
| Patient 1 | Bloody Emesis | Severe | No | Recovered | Alive |
| Patient 2 | GI Bleeding | Severe | No | Recovered | Dead |
| | Vomiting (Coffee Ground) | Moderate | No | Recovered | |
| | Rectal Bleeding | Moderate | No | Recovered | |
| | Recurring GI Bleeding | Severe | No | Recovered | |
| **0.025 mg/kg/hr ala-TFPI** | | | | | |
| None | | | | | |
| **0.05 mg/kg/hr ala-TFPI** | | | | | |
| Patient 3 | Upper Gastrointestinal Bleed | Mild | Remotely | Recovered | Alive |
| **0.1 mg/kg/hr ala-TFPI** | | | | | |
| Patient 4 | Epistaxis | Moderate | Possibly | Recovered | Dead |
| | Gastrointestinal bleed | Moderate | Possibly | Recovered | |
| | Gross hematuria | Moderate | Probably | Persistent | |

**Example 5.** Mortality Rates Across Patient Subgroups

**[118]** Mortality rates were analyzed for different subgroups of patients in the Phase II low-dose ala-TFPI study described in Example 4. The results are summarized in Table 13. Results are compared for all placebo patients vs. all ala-TFPI patients (both 0.025 and 0.050 mg/kg/hr dose rates).

**Table 11.** 28 Day All Cause Mortality Rates Across Subgroups of Septic Patients Treated with Low Dose Ala-TFPI

| **Subgroup** | **All Placebo** n = 69 | **All Ala-TFPI** n = 141 |
|---|---|---|
| APACHE II < 20 | 17% (4/24) | 11% (4/35) |
| APACHE II ≥ 20 | 49% (22/45) | 37% (39/106) |
| No Shock | 30% (7/23) | 18% (9/50) |
| Shock | 41% (19/46) | 37% (34/91) |
| No ARDS | 18% (6/33) | 22% (13/60) |
| ARDS | 57% (20/35) | 37% (30/81) |
| Baseline IL-6 < 1000 pg/ml | 29% (15/52) | 27% (27/101) |
| Baseline IL-6 ≥ 1000 pg/ml | 62% (8/13) | 41% (16/39) |
| Baseline Lab INR < 1.2 | 29% (6/21) | 26% (11/43) |
| Baseline Lab INR ≥ 1.2 | 42% (20/48) | 33% (32/98) |

[119] The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions that may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating sepsis comprising:

administering TFPI or a TFPI analog to a patient who has sepsis or who is at risk of becoming septic by continuous intravenous infusion at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period at least about 72 hours.

2. The method of claim 1 wherein said TFPI analog is non-glycosylated ala-TFPI.

3. The method of claim 1 wherein said TFPI analog comprises a first Kunitz domain consisting of amino acids 19-89 of SEQ ID NO:1.

4. The method of claim 3 wherein said TFPI analog further comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

5. The method of clam 1 wherein said TFPI analog comprises amino acids 1-160 of SEQ ID NO:1.

6. The method of claim 1 wherein said TFPI analog comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

7. The method of claim 1 wherein said dose rate is equivalent to administration of reference ala-TFPI at a dose rate from about 0.010 to about 0.045 mg/kg/hr.

8. The method of claim 7 wherein said TFPI analog is non-glycosylated ala-TFPI.

9. The method of claim 7 wherein said dose rate is equivalent to administration of reference ala-TFPI at a dose rate of about 0.025 mg/kg/hr.

10. The method of claim 9 wherein said TFPI analog is non-glycosylated ala-TFPI.

11. The method of claim 1 wherein said administration period at least about 96 hours.

12. The method of claim 11 wherein said TFPI analog is non-glycosylated ala-TFPI.

13. The method of claim 11 wherein said dose rate is administered to provide a total dose equivalent to administration of reference ala-TFPI at a total dose from about 0.024 to about 4.8 mg/kg.

14. The method of claim 13 wherein said TFPI analog is non-glycosylated ala-TFPI.

15. The method of claim 11 wherein said TFPI or TFPI analog is administered at a dose rate equivalent to administration of reference ala-TFPI at a dose rate of about 0.025 mg/kg/hr.

16. The method of claim 15 wherein said TFPI analog is non-glycosylated ala-TFPI.

17. The method of claim 1 wherein said dose rate is administered to provide a daily dose equivalent to administration of reference ala-TFPI at a daily dose from about 0.006 mg/kg to about 1.2 mg/kg.

18. The method of claim 17 wherein said TFPI analog is non-glycosylated ala-TFPI.

19. The method of claim 1 wherein said TFPI or said TFPI analog is administered to a patient having a baseline International Normalized Ratio (INR) of at least about 1.2.

20. The method of claim 19 wherein said TFPI analog is non-glycosylated ala-TFPI.

21. The method of claim 1 further comprising terminating administering said TFPI or TFPI analog when said patient has an INR either exceeding a baseline INR by at least 20% or having a value of at least about 2.5.

22. The method of claim 21 wherein said TFPI analog is non-glycosylated ala-TFPI.

23. The method of claim 1 wherein said patient has an APACHE II score of at least 20.

24. The method of claim 23 wherein said TFPI analog is non-glycosylated ala-TFPI.

25. The method of claim 1 wherein said patient has a baseline plasma IL-6 concentration of at least about 1000 pg/ml.

26. The method of claim 25 wherein said TFPI analog is non-glycosylated ala-TFPI.

27. The method of claim 1 wherein said patient is suffering from shock.

28. The method of claim 27 wherein said TFPI analog is non-glycosylated ala-TFPI.

29. The method of claim 1 wherein said patient is suffering from ARDS.

30. The method of claim 29 wherein said TFPI analog is non-glycosylated ala-TFPI.

31. The method of claim 1 wherein said patient has a pulmonary score, an ICU score, or a multiple organ dysfunction score that increases during said administration period.

32. The method of claim 31 wherein said TFPI analog is non-glycosylated ala-TFPI.

33. The method of claim 1 wherein said TFPI or TFPI analog is prepared from a lyophilized composition comprising TFPI or a TFPI analog.

34. The method of claim 33 wherein said TFPI analog is non-glycosylated ala-TFPI.

35. The method of claim 1 wherein said TFPI or TFPI analog is administered as a formulation comprising arginine.

36. The method of claim 35 wherein said TFPI analog is non-glycosylated ala-TFPI.

37. The method of claim 1 wherein said TFPI or TFPI analog is administered as a formulation comprising citrate.

38. The method of claim 37 wherein said TFPI analog is non-glycosylated ala-TFPI.

39. The method of claim 1 wherein said TFPI or TFPI analog has a concentration of about 0.15 mg/ml in a formulation comprising about 300 mM arginine hydrochloride and about 20 mM sodium citrate and having a pH of about 5.5.

40. The method of claim 39 wherein said TFPI analog is non-glycosylated ala-TFPI.

41. The method of claim 1 further comprising administering, during or within 24 hours of said administration period, an additional agent selected from the group consisting of an antibiotic, an antibody, an endotoxin antagonist, a tissue factor analog having anticoagulant activity, an immunostimulant, a cell adhesion blocker, heparin, BPI protein, an IL-1 antagonist, pafase (PAF enzyme inhibitor), a TNF inhibitor, an IL-6 inhibitor, and an inhibitor of complement.

42. The method of claim 41 wherein said TFPI analog is non-glycosylated ala-TFPI.

43. The method of claim 41 wherein said additional agent is an antibody, wherein said antibody binds specifically to an antigen selected from the group consisting of TNF, IL-6, and M-CSF.

44. The method of claim 43 wherein said TFPI analog is non-glycosylated ala-TFPI.

45. A prophylactic method for decreasing the risk and severity of sepsis, said method comprising administering TFPI or a TFPI analog to a patient susceptible to sepsis or suspected of being septic by continuous intravenous infusion at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period of at least about 72 hours.

46. The method of claim 45 wherein said TFPI analog is non-glycosylated ala-TFPI.

47. The method of claim 45 wherein said TFPI analog comprises a first Kunitz domain consisting of amino acids 19-89 of SEQ ID NO:1.

48. The method of claim 47 wherein said TFPI analog further comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

49. The method of claim 45 wherein said TFPI analog comprises amino acids 1-160 of SEQ ID NO:1.

49. The method of claim 45 wherein said TFPI analog comprises a second Kunitz domain consisting of amino acids 90-160 of SEQ ID NO:1.

50. The method of claim 45 wherein said dose rate is equivalent to administration of reference ala-TFPI at a dose rate from about 0.010 to about 0.045 mg/kg/hr.

51. The method of claim 50 wherein said TFPI analog is non-glycosylated ala-TFPI.

52. The method of claim 50 wherein said dose rate is equivalent to administration of reference ala-TFPI at a dose rate of about 0.025 mg/kg/hr.

53. The method of claim 52 wherein said TFPI analog is non-glycosylated ala-TFPI.

54. The method of claim 45 wherein said administration period is at least about 96 hours.

55. The method of claim 54 wherein said TFPI analog is non-glycosylated ala-TFPI.

56. The method of claim 54 wherein said dose rate equivalent to administration of reference ala-TFPI at a dose rate of about 0.025 mg/kg/hr.

57. The method of claim 56 wherein said TFPI analog is non-glycosylated ala-TFPI.

58. A method for prophylactically and therapeutically treating acute inflammation, including sepsis and septic shock, said method comprising administering to a patient (i) a continuous intravenous infusion of TFPI or a TFPI analog at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr and (ii) an

additional agent selected from the group consisting of an antibiotic, a monoclonal antibody, a cytokine inhibitor, and a complement inhibitor.

59. The method of claim 58 wherein said TFPI analog is non-glycosylated ala-TFPI.

60. A method for treating a disease state not associated with DIC and in which TNF, IL-1, or another cytokine up-regulates tissue factor, said method comprising administering to a patient a continuous intravenous infusion of an agent selected from the group consisting of TFPI or a TFPI analog at a dose rate equivalent to administration of reference ala-TFPI at a dose rate from about 0.00025 to about 0.050 mg/kg/hr for an administration period of at least about 72 hours.

61. The method of claim 60 wherein said TFPI analog is non-glycosylated ala-TFPI.

62. The method of claim 60 wherein said disease state is chronic or acute inflammation.

63. The method of claim 62 wherein said TFPI analog is non-glycosylated ala-TFPI.

64. The method of claim 60 wherein said patient has a plasma concentration of IL-6 that is reduced during said administration period.

65. The method of claim 64 wherein said TFPI analog is non-glycosylated ala-TFPI.