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(71) Applicant(s)
Xoma Corporation

(72) Inventor(s)
Patrick J. Scannon; Nancy Wedel

(74) Agent/Attorney
BALDWIN SHELSTON WATERS, Level 21, 60 Margaret Street, SYDNEY NSW 2000

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(54) Title: THERAPEUTIC USES OF BPI PROTEIN PRODUCTS IN HUMANS WITH HEMORRHAGE DUE TO TRAUMA (57) Abstract Methods and materials for the treatment of humans suffering from hemorrhage due to trauma are provided, in which therapeutically effective amounts of BPI protein products are administered.		

THERAPEUTIC USES OF BPI PROTEIN PRODUCTS IN HUMANS WITH HEMORRHAGE DUE TO TRAUMA

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BACKGROUND OF THE INVENTION

The present invention relates generally to methods and materials for treating humans suffering from hemorrhage due to trauma, by administration of bactericidal/permeability-increasing (BPI) protein products.

10 Acute traumatic hemorrhage, generally requiring immediate surgical intervention, is a major contributor to morbidity and mortality in the U.S. [Bickell et al., *New Eng. J. Med.*, 331:1105-1109 (1994), Tran et al., *Surgery*, 114:21-30 (1993).] In 1982, there were approximately 165,000 deaths in the U.S. due to trauma, with at least two additional cases of permanent disability for each death. About 50% of these traumatic deaths occur immediately, due to direct injury to the
15 central nervous system, heart, or one of the major blood vessels. Additional early deaths, approximately 30%, occur within several hours after injury, usually due to uncontrolled hemorrhage. The remaining 20% of deaths are so-called "late deaths", occurring during days to weeks after injury, due to complications from the traumatic hemorrhage that include infection or multiple organ system failure (MOSF) in about
20 80% of the cases. [Trunkey, *Sci. Am.*, 249:28-35 (1983), Trunkey, *New Eng. J. Med.*, 324:1259-1263 (1991).]

Among those patients who survive the immediate resuscitative and surgical interventions, approximately 10-40% suffer from a variety of morbidities, including, for example, systemic inflammation, wound infections, pneumonia, sepsis,
25 respiratory failure, renal failure, coagulopathy, and pancreatitis. Hemorrhage and transfusion requirements may be specifically linked to increased risk of postoperative infection, respiratory complications, and multiorgan system failure [Agarwal et al., *Arch. Surg.*, 128:171-177 (1993), Duke et al., *Arch. Surg.*, 128:1125-1132 (1993), Tran et al., *supra*].

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The causes of these complications from traumatic hemorrhage are multifactorial and interrelated. Many morbidities may be related to systemic



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inflammation following injury. It has also been hypothesized that physical trauma to tissue, direct tissue hypoperfusion, and translocation of endogenous bacteria and absorption of endotoxin from the gut lumen (due to hypoperfusion and/or other injury to the gastrointestinal tract) may play a role in the pathogenesis of these complications. The relevance of these proposed factors in the pathophysiology of the morbidities and late deaths associated with acute hemorrhagic shock in humans, however, is not clear.

Although acute traumatic hemorrhage is one potential cause of hypovolemic shock (*i.e.*, shock due to decreased intravascular volume), there are numerous other potential causes, such as internal bleeding, *e.g.*, gastrointestinal hemorrhage, intraperitoneal or retroperitoneal hemorrhage, hemorrhage into the femoral compartment, intrathoracic hemorrhage, aortic dissection and ruptured aortic aneurysm; excessive fluid loss due to, *e.g.*, severe vomiting due to an intestinal or pyloric obstruction, severe diarrhea, sweating, dehydration, excessive urination (due to diabetes mellitus, diabetes insipidus, excessive diuretics, or the diuretic phase of acute renal failure), peritonitis, pancreatitis, planchnic ischemia, gangrene, burns; vasodilation due to, *e.g.*, nervous system damage, anesthesia, ganglionic and adrenergic blockers, barbiturate overdose, poisons; and metabolic, toxic, or humoral vasodilatation, such as acute adrenal insufficiency, or an anaphylactic reaction. Other causes of shock unrelated to circulatory volume loss include cardiogenic shock (*e.g.*, acute myocardial infarction, cardiac tamponade) and obstructive shock (*e.g.*, acute pulmonary embolism). [See, *e.g.*, *Manual of Medical Therapeutics*, 28th ed., Ewald et al., eds., Little, Brown and Company, Boston (1995); *Cecil's Textbook of Medicine*, 17th ed., Wyngaarden et al., eds., W.B. Saunders Co., Philadelphia (1985).]

As outlined below in Table I below, a normal individual can rapidly lose up to 20 per cent of the blood volume without any signs or symptoms. Limited signs of cardiovascular distress appear with losses up to 30 per cent of the blood volume, but signs and symptoms of hypovolemic shock generally appear when the blood loss exceeds 30 to 40 per cent of the blood volume.

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Table I

Percentage of Blood Volume Lost	Amount Lost (ml)	Clinical Manifestations
10-20%	500-1000	Usually none, perhaps mild postural hypotension and tachycardia in response to exercise; vasovagal syncope may occur in 5% of cases
20-30%	1000-1500	Few changes supine; light-headedness and hypotension commonly occur when upright; marked tachycardia in response to exertion
30-40%	1500-2000	Blood pressure, cardiac output, central venous pressure, and urine volume are reduced even when supine; thirst, shortness of breath, clammy skin, sweating, clouding of consciousness and rapid, thready pulse may be noted
40-50%	2000-2500	Severe shock, often resulting in death

The patient is frequently oliguric, with a urinary output of less than 20 mL per hour. Frequently, the physical findings follow a progressive pattern as shock evolves from the early compensated phase to the advanced stages. In Stage I, physiologic compensatory mechanisms, such as increased cardiac output or elevated systemic vascular resistance, are effective and minimal clinical symptoms and signs are observed. In Stage II, these mechanisms cannot effectively compensate for the blood volume loss, and the patient may exhibit hypotension, tachycardia, and hyperventilation. The decreased perfusion of vital organs can result in an altered mental state ranging from agitation to stupor to coma, reduced urinary output, and myocardial ischemia (in patients with coronary artery disease). The external appearance of the patient also reflects excessive sympathetic discharge, with cyanosis, coldness, and clamminess of the skin. In Stage III, which may be irreversible, the excessive and prolonged reduction of tissue perfusion leads to significant alterations in cellular membrane function, aggregation of blood corpuscles, and "sludging" in the capillaries. The vasoconstriction which has taken place in the less vital organs in order to maintain blood pressure is now excessive and has reduced flow to such an extent that cellular damage occurs.

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Following traumatic hemorrhage, conventional therapy is directed at stopping the hemorrhage, combating shock, and restoring the blood volume. Prompt fluid resuscitation is preferably given through large-bore catheters placed in large peripheral veins. The pneumatic antishock garment, with sequential inflation of legs and abdominal compartments to 15-40 mm Hg, may temporally stabilize patients by increasing peripheral systemic vascular resistance. Restoration of the blood volume may be achieved by intravenous infusion of electrolyte solutions; colloid solutions of plasma protein, albumin, or dextran; or fresh whole blood. In the emergency situation, electrolyte solutions, albumin, or dextran are preferred over fresh whole blood because of the large amounts of fluid required, the possible delay in transfusion if typing and cross-matching are performed, and the possibility of allergic transfusion reactions. When shock is due to hemorrhage, packed red blood cells should be given as soon as feasible. When hemorrhage is massive, type-specific unmatched blood can be given safely. Rarely, type O blood may be needed.

Rapid infusion of Ringer's lactated or normal saline solution is the most widely used fluid therapy following hemorrhage. An initial infusion of two to three times the volume of the estimated blood loss is administered. Because these solutions are rapidly distributed throughout the intravascular and extravascular compartments, they must be supplemented with colloid solutions. When large volumes of electrolyte solutions are infused, patients often develop peripheral edema and elderly patients may develop pulmonary edema.

The colloidal preparations in wide use include a 6 per cent solution of high molecular weight dextran (dextran 70), a 10 per cent solution of low molecular weight dextran (dextran 40), and a 5 per cent solution of albumin in normal saline. Infusions of dextran 70 produce an initial volume effect slightly greater than the amount infused. Dextran 70 is slowly cleared over one to two days, allowing time for normal physiologic mechanisms to replace the volume lost. Dextran 40 has the advantage of an initial volume effect of nearly twice the amount infused. The lower molecular weight material is more rapidly cleared, however, and the volume-expanding effect is dissipated by 24 hours, before normal volume replacement mechanisms are maximal. Acute renal failure has occurred in a few patients receiving dextran 40. With either dextran solution, volumes in excess of one liter

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may interfere with platelet adhesiveness and the normal coagulation cascade. A solution of 5 per cent albumin in normal saline has the advantage of producing a known volume effect in the hypovolemic patient, but this preparation is relatively costly and time-consuming to prepare. A hypertonic albumin preparation containing 5 120 mEq of sodium lactate, 120 mEq of sodium chloride, and 12.5 grams of albumin per liter provides a predictable volume effect and minimizes interstitial fluid leakage. Use of hypertonic solutions requires careful monitoring of arterial and central venous pressures to avoid fluid overload. Coexisting problems such as congestive heart failure, valvular heart disease, myocardial ischemia, or renal insufficiency must be 10 carefully monitored, and invasive hemodynamic monitoring must be considered during acute management. Associated coagulopathy and electrolyte imbalance must also be corrected.

BPI is a protein isolated from the granules of mammalian polymorphonuclear leukocytes (PMNs or neutrophils), which are blood cells essential 15 in the defense against invading microorganisms. Human BPI protein has been isolated from PMNs by acid extraction combined with either ion exchange chromatography [Elsbach, *J. Biol. Chem.*, 254:11000 (1979)] or *E. coli* affinity chromatography [Weiss, et al., *Blood*, 69:652 (1987)]. BPI obtained in such a manner is referred to herein as natural BPI and has been shown to have potent 20 bactericidal activity against a broad spectrum of gram-negative bacteria. The molecular weight of human BPI is approximately 55,000 daltons (55 kD). The amino acid sequence of the entire human BPI protein and the nucleic acid sequence of DNA encoding the protein have been reported in Figure 1 of Gray et al., *J. Biol. Chem.*, 264:9505 (1989), incorporated herein by reference. The Gray et al. amino acid 25 sequence is set out in SEQ ID NO: 1 hereto. U.S. Patent No. 5,198,541 discloses recombinant genes encoding and methods for expression of BPI proteins, including BPI holoprotein and fragments of BPI.

BPI is a strongly cationic protein. The N-terminal half of BPI accounts for the high net positive charge; the C-terminal half of the molecule has a net charge 30 of -3. [Elsbach and Weiss (1981), *supra*.] A proteolytic N-terminal fragment of BPI having a molecular weight of about 25 kD possesses essentially all the anti-bacterial efficacy of the naturally-derived 55 kD human BPI holoprotein. [Ooi et al., *J. Bio.*

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Chem., 262: 14891-14894 (1987)]. In contrast to the N-terminal portion, the C-terminal region of the isolated human BPI protein displays only slightly detectable anti-bacterial activity against gram-negative organisms. [Ooi et al., *J. Exp. Med.*, 174:649 (1991).] An N-terminal BPI fragment of approximately 23 kD, referred to as "rBPI₂₃," has been produced by recombinant means and also retains anti-bacterial activity against gram-negative organisms. Gazzano-Santoro et al., *Infect. Immun.* 60:4754-4761 (1992).

The bactericidal effect of BPI has been reported to be highly specific to gram-negative species, e.g., in Elsbach and Weiss, *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin et al., Chapter 30, Raven Press, Ltd. (1992). The precise mechanism by which BPI kills gram-negative bacteria is not yet completely elucidated, but it is believed that BPI must first bind to the surface of the bacteria through electrostatic and hydrophobic interactions between the cationic BPI protein and negatively charged sites on LPS. In susceptible gram-negative bacteria, BPI binding is thought to disrupt LPS structure, leading to activation of bacterial enzymes that degrade phospholipids and peptidoglycans, altering the permeability of the cell's outer membrane, and initiating events that ultimately lead to cell death. [Elsbach and Weiss (1992), *supra*]. LPS has been referred to as "endotoxin" because of the potent inflammatory response that it stimulates, i.e., the release of mediators by host inflammatory cells which may ultimately result in irreversible endotoxic shock. BPI binds to lipid A, reported to be the most toxic and most biologically active component of LPS.

BPI protein has never been used previously for the treatment of humans suffering from hemorrhage due to trauma or the shock associated with traumatic blood loss (*i.e.*, hypovolemic shock). Bahrami et al., presentation at Vienna International Endotoxin Society Meeting, August, 1992, report the administration of BPI protein to rats subjected to hemorrhage. Yao et al., *Ann. Surg.*, 221:398-405 (1995), report the administration of rBPI₂₁ (described *infra*) to rats subjected to prolonged hemorrhagic insult for 180 minutes followed by resuscitation. U.S. Patent Nos. 5,171,739, 5,089,724 and 5,234,912 report the use of BPI in various *in vitro* and *in vivo* animal model studies asserted to be correlated to methods of treating endotoxin-related diseases, including endotoxin-related shock. In co-owned, co-

pending Australian application No. 16944/95, incorporated herein by reference, the administration of BPI protein product to humans with endotoxin in circulation was described. [See also, von der Mohlen et al., *J. Infect. Dis.* 172:144-151 (1995); von der Mohlen et al., *Blood* 85: 3437-3443 (1995); de Winter et al., *J. Inflam.* 45:193-
5 206(1995)]. In co-owned, co-pending Australian application No. 30043/97, the administration of BPI protein product to humans suffering from severe meningococemia was described.

In spite of treatment with antibiotics and state-of-the-art medical intensive care therapy, human mortality and morbidities associated with haemorrhage due to trauma
10 remain significant and unresolved by current therapies. New therapeutic methods are needed that could reduce or ameliorate the adverse events and improve the clinical outcome of such patients.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common
15 general knowledge in the field.

SUMMARY OF THE INVENTION

The present invention provides novel methods for treating humans suffering from haemorrhage due to trauma, involving the administration of BPI protein products to provide clinically verifiable alleviation of the adverse effects of, or complications
20 associated with, this disease state, including mortality and complications or morbidities. According to the invention, BPI protein products such as rBPI₂ are administered to humans suffering from acute traumatic haemorrhage in amounts sufficient to reduce or prevent mortality and/or to reduce the incidence (ie occurrence) or severity of complications or morbidities, including infection (eg, surgical site infection) or organ



dysfunction (eg, disseminated intravascular coagulation, acute respiratory distress syndrome, acute renal failure, or hepatobiliary dysfunction).

According to a first aspect the present invention provides a method of treating a human suffering from haemorrhage due to trauma comprising the step of administering a
5 therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to said human.

According to a second aspect the present invention provides the use of a BPI protein product in the preparation of a medicament for the treatment of humans suffering from haemorrhage due to trauma.

10 According to a third aspect the present invention provides a method of treating a pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma comprising the step of administering a therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to said human.

15 According to a fourth aspect the present invention provides use of a BPI protein product in the manufacture of a medicament for treating a pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense
20 of "including, but not limited to".

Also contemplated is use of a BPI protein product in the preparation of a medicament for the treatment of humans suffering from haemorrhage due to trauma.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following _____



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detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the incidence of adverse events in rBPI₂₁ and placebo
5 treatment groups.

DETAILED DESCRIPTION

Acute hemorrhage due to trauma is a life-threatening condition with significant mortality and morbidities despite state-of-the-art medical intensive care. The administration of BPI protein products to humans suffering from hemorrhage due
10 to acute traumatic injury (such as penetrating and/or blunt trauma) is expected to effectively decrease mortality and reduce the incidence (*i.e.*, occurrence) or severity of complications or morbidities associated with or resulting from hemorrhage due to trauma. Complications include infection (e.g., in surgical sites, wounds, organs, anatomical spaces, the bloodstream, the urinary tract, or pneumonia) or organ
15 dysfunction (e.g., disseminated intravascular coagulation, acute respiratory distress syndrome (ARDS), acute renal failure, or hepatobiliary dysfunction), and may include serious complications. An additional complication may be pulmonary dysfunction, which includes ARDS and pneumonia. These unexpected effects on the mortality and complications associated with and resulting from hemorrhage due to trauma indicate
20 that BPI protein products effectively interfere with or block a number of the multiple poorly-understood pathophysiologic processes that have led to poor outcomes in this condition. BPI protein products may be used as adjunctive therapy in the treatment or prevention of organ dysfunction and serious infections. BPI protein products are expected to provide beneficial effects for patients suffering from hemorrhage due to
25 trauma, such as reduced injury severity score, reduced length of time on ventilatory support and inotropic (vasoactive) therapy, reduced duration or severity of associated coagulopathy, reduced stay in the ICU, reduced stay in the hospital overall, and reduced incidence and duration of complications such as coagulopathy, respiratory failure, renal failure, hepatic failure, coma or altered mental state, adrenal cortical
30 necrosis, and severe infection, including

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in wounds, organs, anatomical spaces, the bloodstream, the urinary tract, or pneumonia.

Therapeutic compositions comprising BPI protein product may be administered systemically or topically. Systemic routes of administration include oral, intravenous, intramuscular or subcutaneous injection (including into a depot for long-term release), intraocular and retrobulbar, intrathecal, intraperitoneal (e.g. by intraperitoneal lavage), intrapulmonary using aerosolized or nebulized drug, or transdermal. The preferred route is intravenous administration. When given parenterally, BPI protein product compositions are generally injected in doses ranging from 1 $\mu\text{g/kg}$ to 100 mg/kg per day, preferably at doses ranging from 0.1 mg/kg to 20 mg/kg per day, more preferably at doses ranging from 1 to 20 mg/kg/day and most preferably at doses ranging from 2 to 10 mg/kg/day. Treatment may be initiated immediately after the trauma or within a time period subsequent to the trauma (including, e.g., within 6, 12 or 24 hours after trauma, or within a clinically reasonable time period determined by the treating physician, for example, 48 to 72 hours after trauma). Presently preferred is a continuous intravenous infusion of BPI protein product at a dose of 4 to 6 mg/kg/day, continuing for 48 to 72 hours. The treatment may continue by continuous infusion or intermittent injection or infusion, at the same, reduced or increased dose per day for, e.g., 1 to 3 days, and additionally as determined by the treating physician. Alternatively, BPI protein products are administered intravenously by an initial bolus followed by a continuous infusion. One such regimen is a 1 to 20 mg/kg intravenous bolus of BPI protein product followed by intravenous infusion at a dose of 1 to 20 mg/kg/day, continuing for up to one week. Another such dosing regimen is a 2 to 10 mg/kg initial bolus followed by intravenous infusion at a dose of 2 to 10 mg/kg/day, continuing for up to 72 hours. Topical routes include administration in irrigation fluids for, e.g., irrigation of wounds, or intrathoracic or intraperitoneal cavities. Other topical routes include administration in the form of salves, ophthalmic drops, ear drops, or medicated shampoos. For example, for topical administration in drop form, about 10 to 200 μL of a BPI protein product composition may be applied one or more times per day as determined by the treating physician. Those skilled in the art can readily optimize effective dosages and administration regimens for therapeutic compositions comprising

BPI protein product, as determined by good medical practice and the clinical condition of the individual patient.

As used herein, "BPI protein product" includes naturally and recombinantly produced BPI protein; natural, synthetic, and recombinant biologically active

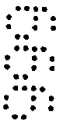
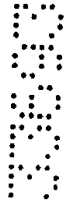
5 polypeptide fragments of BPI protein; biologically active polypeptide variants of BPI protein or fragments thereof, including hybrid fusion proteins and dimers; biologically active polypeptide analogs of BPI protein or fragments or variants thereof, including cysteine-substituted analogs; and BPI-derived peptides. The BPI protein products administered according to this invention may be generated and/or isolated by any means
10 known in the art. US Patent No.5,198,541, the disclosure of which is incorporated herein by reference, discloses recombinant genes encoding and methods for expression of BPI proteins including recombinant BPI holoprotein, referred to as rBPI₅₀ (or rBPI) and recombinant fragments of BPI. Co-owned, copending Australian application No. 43820/93 and PCT application No.93/04752, which are both incorporated herein by
15 reference, disclose novel methods for the purification of recombinant BPI protein products expressed in and secreted from genetically transformed mammalian host cells in culture and discloses how one may produce large quantities of recombinant BPI products suitable for incorporation into stable, homogeneous pharmaceutical preparations.

20 Biologically active fragments of BPI (BPI fragments) include biologically active molecules that have the same or similar amino acid sequence as a natural human BPI holoprotein, except that the fragment molecule lacks amino-terminal amino acids, internal amino acids, and/or carboxy-terminal amino acids of the holoprotein.

Nonlimiting examples of such fragments include a N-terminal fragment of natural



human BPI of approximately 25 kD, described in Ooi et al., *J. Exp. Med.*, 174:649 (1991), and the recombinant expression product of DNA encoding N-terminal amino acids from 1 to about 193 or 199 of natural human BPI, described in Gazzano-Santoro et al., *Infect. Immun.* 60:4754-4761 (1992), and referred to as rBPI₂₃. In that publication, an
5 expression vector was used as a source of DNA encoding a recombinant expression product (rBPI₂₃) having the 31-residue _____



signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in Figure 1 of Gray et al., *supra*, except that valine at position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). Recombinant holoprotein (rBPI₅₀) has
5 also been produced having the sequence (SEQ ID NOS: 1 and 2) set out in Figure 1 of Gray et al., *supra*, with the exceptions noted for rBPI₂₃ and with the exception that residue 417 is alanine (specified by GCT) rather than valine (specified by GTT). Other examples include dimeric forms of BPI fragments, as described in co-owned and co-pending U.S. Patent Application Serial No. 08/212,132, filed March 11, 1994,
10 and corresponding PCT Application No. PCT/US95/03125, the disclosures of which are incorporated herein by reference. Preferred dimeric products include dimeric BPI protein products wherein the monomers are amino-terminal BPI fragments having the N-terminal residues from about 1 to 175 to about 1 to 199 of BPI holoprotein. A particularly preferred dimeric product is the dimeric form of the BPI fragment having
15 N-terminal residues 1 through 193, designated rBPI₄₂ dimer.

Biologically active variants of BPI (BPI variants) include but are not limited to recombinant hybrid fusion proteins, comprising BPI holoprotein or biologically active fragment thereof and at least a portion of at least one other polypeptide, and dimeric forms of BPI variants. Examples of such hybrid fusion
20 proteins and dimeric forms are described by Theofan et al. in co-owned, copending U.S. Patent Application Serial No. 07/885,911, and a continuation-in-part application thereof, U.S. Patent Application Serial No. 08/064,693 filed May 19, 1993 and corresponding PCT Application No. US93/04754 filed May 19, 1993, which are all incorporated herein by reference and include hybrid fusion proteins comprising, at the
25 amino-terminal end, a BPI protein or a biologically active fragment thereof and, at the carboxy-terminal end, at least one constant domain of an immunoglobulin heavy chain or allelic variant thereof. Similarly configured hybrid fusion proteins involving part or all Lipopolysaccharide Binding Protein (LBP) are also contemplated for use in the present invention.

30 Biologically active analogs of BPI (BPI analogs) include but are not limited to BPI protein products wherein one or more amino acid residues have been replaced by a different amino acid. For example, co-owned, copending



Australian patent application No. 61702/94 and corresponding PCT application

No.US94/01235, the disclosures of which are incorporated herein by reference, discloses polypeptide analogs of BPI and BPI fragments wherein a cysteine residue is replaced by a different amino acid. A preferred BPI protein product described by this application is

5 the expression product of DNA encoding from amino acid 1 to approximately 193 or 199 of the N-terminal amino acids of BPI holoprotein, but wherein the cysteine at residue number 132 is substituted with alanine and is designated rBPI₂₁Δcys or rBPI₂₁. Other examples include dimeric forms of BPI analogs; eg co-owned and co-pending Australian patent application No. 19969/95, and corresponding PCT application

10 No.PCT/US95/03125, the disclosures of which are incorporated herein by reference.

Other BPI protein products useful according to the methods of the invention are peptides derived from or based on BPI produced by recombinant or synthetic means (BPI-derived peptides), such as those described in co-owned and co-pending Australian patent application 53675/94 and in co-owned and copending PCT application No.

15 PCT/US94/10427, which corresponds to Australian application No. 31981/95 and PCT application No. US94/02465 filed March 11, 1994, which corresponds to Australian patent Nos. 681453, 694108 and 684503, the disclosures of all of which are incorporated herein by reference.

Presently preferred BPI protein products include recombinantly-produced N-
20 terminal fragments of BPI, especially those having a molecular weight of approximately between 21 to 25 kD such as rBPI₂₃ or rBPI₂₁, or dimeric forms of these N-terminal fragments (eg rBPI₄₂ dimer). Additionally, preferred BPI protein products include rBPI₃₀ and BPI-derived peptides. Particularly preferred is rBPI₂₁.



The administration of BPI protein products is preferably accomplished with a pharmaceutical composition comprising a BPI protein product and a pharmaceutically acceptable diluent, adjuvant, or carrier. The BPI protein product may be administered without or in conjunction with known surfactants, other chemotherapeutic agents or additional known anti-microbial agents. One pharmaceutical composition containing BPI protein products (e.g., rBPI₅₀, rBPI₂₃) comprises the BPI protein product at a concentration of 1 mg/ml in citrate buffered saline (5 or 20 mM citrate, 150 mM NaCl, pH 5.0) comprising 0.1% by weight of poloxamer 188 (Pluronic F-68, BASF Wyandotte, Parsippany, NJ) and 0.002% by weight of polysorbate 80 (Tween 80, ICI Americas Inc., Wilmington, DE). Another pharmaceutical composition containing BPI protein products (e.g., rBPI₂₁) comprises the BPI protein product at a concentration of 2 mg/mL in 5 mM citrate, 150 mM NaCl, 0.2% poloxamer 188 and 0.002% polysorbate 80. Such combinations are described in co-owned, co-pending PCT Application No. US94/01239 filed February 2, 1994, which corresponds to U.S. Patent Application Ser. No. 08/190,869 filed February 2, 1994 and Australian Patent application No. 61330/94, the disclosures of all of which are incorporated herein by reference.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 addresses the effect of BPI protein product administration in humans on the mortality and complications associated with hemorrhage due to trauma.

EXAMPLE 1

Clinical Study Protocol - Therapeutic Effects of BPI Protein Product

A human clinical study was designed to examine the effect of an exemplary BPI protein product, rBPI₂₁, in the treatment of patients with acute hemorrhage due to trauma. Thus, a multicenter, randomized, double-blind, placebo-controlled trial was implemented comparing placebo treatment and rBPI₂₁ treatment given over 48 hours in patients with acute hemorrhage due to trauma. Approximately 400 patients admitted to the emergency department with acute hemorrhage due to trauma and requiring transfusion of at least two units of blood were randomized in



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a 1:1 ratio for treatment with either rBPI₂₁ or placebo. In addition to standard therapy, each patient received by continuous intravenous infusion either rBPI₂₁ at 8 mg/kg over 48 hours (4 mg/kg/day x 2 days) or the equivalent volume of placebo. In most instances the weight of the patient in kilograms was determined as a best estimate.

Efficacy was monitored from Day 1 to Day 15 by following patients for development of complications, such as impaired organ function and infection, and for survival. Safety was monitored by pre-treatment and serial post-treatment testing of chemistries and hematology parameters, as well as daily assessments for adverse events through Day 15. A final assessment of survival and adverse complications occurred on Day 29.

Patients brought to the hospital with acute hemorrhage due to trauma were selected for enrollment in the study if they met the following inclusion and exclusion criteria. Inclusion criteria were: (1) age 18 (or age of consent) to 75 years, inclusive; (2) patient suffering from acute hemorrhage secondary to trauma; (3) study drug given within 6 or 12 hours of occurrence of the traumatic event (if precise time of event was unknown, best estimate was provided); (4) patient requires and has begun to receive a second unit of packed red blood cells; and (5) patient provides verbal informed consent or next of kin provides written informed consent.

Exclusion criteria were: (1) a Triage Revised Trauma Score (TRTS, scale 0-12) less than 2.0 upon admission to the Emergency Department, see Table II below [Champion et al., *Crit. Care Med.*, 9(9):672-676 (1981); Greenfield et al., Chapter 10, in *Surgery Scientific Principles and Practices*, J.B. Lippincott Co., Philadelphia, pp. 252-255 (1993)]; (2) severe head trauma (Glasgow Coma Score (GCS) $\leq$ 5 or equivalent evidence), see Table III below [Teasdale et al., *Lancet*, 1: 81 (1974)]; (3) isolated cranial injury; (4) spinal injury with paralysis; (5) burn injuries with at least 20% body surface area with second degree burns; (6) known positive HIV (test not mandatory at entry); (7) known pre-existing renal disease (creatinine $>$ 2.0); (8) known pre-existing cardiac disease (NY Heart Association class greater than III, see Table IV below [Braunwald, in Braunwald et al., *Heart Disease, The Textbook of Cardiovascular Medicine*, 3rd ed., W.B. Saunders Company, Philadelphia, PA, page 12 (1988); *J. Am. Med. Ass'n*, 249:539-544 (1988)]); (9) known pre-existing primary

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or metastatic malignancy in visceral organs; (10) arterial pH (at initial evaluation) < 6.8 or base deficit > 15 (if measured); (11) known current steroid therapy (> 10 mg prednisone/day for > one month); (12) known pre-existing cirrhosis or active hepatitis; (13) pregnancy or lactation; (14) participation in other investigational drug studies (including investigational blood products) within previous 30 days; (15) weight (estimated) greater than 120 kg; and (16) a "do not resuscitate" (DNR) or equivalent order.

Table II
Triage Revised Trauma Score (TRTS)*

10	ASSESSMENT	METHOD	CODING		
	Respiratory Rate (RR)	Count respiratory rate in 15 sec and multiply by 4	10-29 > 29 6-9 1-5 0	= = = = =	4 3 2 1 0
15	Systolic Blood Pressure (SBP)	Measure systolic cuff pressure in either arm by auscultation or palpation	> 89 76-89 50-75 1-49 0	= = = = =	4 3 2 1 0
	Glasgow Coma Score (GCS)	Calculate according to Table III below	Convert GCS to the Following Code:		
			13-15 9-12 6-8 4-5 < 4	= = = = =	4 3 2 1 0

*The TRTS is the sum of the codes for RR, SBP and GCS (range 0-12).

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Table III

Glasgow Coma Scale*

Eye Opening		
5	Spontaneous	4
	Response to sound	3
	Response to pain	2
	Never	1
Motor Response		
10	Obey commands	6
	Localized pain	5
	Normal flexion	4
	(withdrawal)	
15	Abnormal flexion	3
	(decorticate)	
	No response	1
Verbal Response		
20	Oriented	5
	Confused conversation	4
	Inappropriate words	3
	Incomprehensible sounds	2
	None	1

* Scores range from 3 to 15

Table IV
Modified New York Heart Association
Functional Classification

	Class I.	Patients with cardiac disease but with no limitation of physical activity. Ordinary physical activity causes no undue dyspnea, anginal pain, fatigue, or palpitation.
5	Class IIS.	Patients with slight limitation of physical activity. They are comfortable at rest and with moderate exertion. They experience symptoms only with the more strenuous grades of ordinary activity.
	Class IIM.	Patients with moderate limitation of physical ability. They are comfortable at rest and with mild exertion. They experience symptoms with moderate grades of ordinary activity.
	Class III.	Patients with marked limitation of physical activity. They are comfortable at rest but experience symptoms even with the milder forms of ordinary activity.
	Class IV.	Patients with inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present, even at rest, and are intensified by activity.

The following were recorded for all patients randomized to treatment:

- 10 (1) date and estimated time of incident, and date and time of admission to the Emergency Department; (2) for patients randomized and not treated, the reason for not treating; (3) from arrival at hospital until approximately 48 hours post-operatively, date, time, volume, and location that the patient received blood, blood products, and fluids such as packed red blood cells, whole blood, autotransfusion,
- 15 platelets, fresh frozen plasma, crystalloid, or colloid, at locations such as Emergency Department, Operating Room, Post-anesthesia Care Unit, or Surgical Intensive Care Unit; however, if the patient did not undergo surgery, the above items that were applicable were collected during study days 1, 2, and 3; (4) date and time the second unit of blood was administered (which should have preceded surgery, to assure that
- 20 hemorrhage is due to trauma, not surgery), and date and start and stop times of anesthesia; (5) date and start and stop times of surgery, estimated blood loss in operating room, and date and time in post-anesthesia care unit; (6) date and time

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study drug infusion began and ended, volume infused, and reasons for temporary or permanent discontinuation; if applicable, and if discontinued, quantity infused; (7) directed medical history (including extent and nature of injuries, intercurrent diseases, conditions contributing to bleeding, etc.), demographic and directed physical exam information, such as gender, age, weight (estimated or measured), height (estimated or measured), vital signs, physical signs of injury; (8) results of the pregnancy test performed during screening for eligibility of appropriate female patients (all women of child bearing potential, *i.e.*, all women who were not either surgically sterile or documented to be post-menopausal); and (9) results of the TRTS performed during screening for eligibility (including actual measurements).

After transfusion of the second unit of blood was initiated, the investigator administered an unknown test drug from kits in numbered consecutive order. Each kit contained either rBPI₂₁ or placebo. The rBPI₂₁ was supplied as a clear, colorless, sterile non-pyrogenic solution in 10 mL single use glass vials at a concentration of 2 mg/mL in 5 mM sodium citrate/0.15 M sodium chloride buffer, pH 5.0 with 0.2% poloxamer 188 and 0.002% polysorbate 80, containing no preservative. The rBPI₂₁ was stored refrigerated at 2-8°C at all times prior to administration. The placebo was supplied as a clear, colorless sterile non-pyrogenic solution in 10 mL single use glass vials. It was composed of 0.2 mg/mL human serum albumin in 5 mM sodium citrate/0.15 M sodium chloride buffer, pH 5.0, containing no preservative. The placebo was also stored refrigerated at 2-8°C at all times prior to administration. The kit assigned to each patient contained a sufficient number of vials of study medication for all doses for that patient. Each vial contained 10 mL of test article.

The study was administered to two groups ("active" rBPI₂₁ and placebo control) as outlined above. The study medication was brought to room temperature prior to infusion. Throughout the dosing procedure, good aseptic technique for intravenous administration was followed. The study medication was administered by intravenous infusion into a central or peripheral vein over 48 hours. The infusion bag/tubing administration set was completely changed after 24 hours. Suitability of intravenous access was determined by easy withdrawal of blood from the access, as well as easy infusion of intravenous fluids without infiltration. The

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study medication was the sole agent administered in the chosen port during the course of the infusion protocol. The venous access port was not heparinized, but was flushed as necessary with physiologic saline. Any sign of a reaction at a site of infusion was recorded on the patient's case record form and source document as an adverse experience.

5 Patients treated at selected study sites are assessed for: (1) blood levels of rBPI₂₁: blood for the assessment of the rBPI₂₁ level is drawn at the following times (at selected study sites only): prior to the start of the infusion (up to 60 minutes prior to the start of the infusion), the following times (hours) after the start of the infusion; 1, 4, 8, 12, 20, 24, 32, 36, 40, within 15 minutes prior to the completion of the 48 hour infusion, and the following times after completion of the infusion; 7 minutes (48:07), 15 minutes (48:15), 30 minutes (48:30), 1 hour (49:00), 10 3 hours (51:00), 6 hours (54:00), and 24 hours (72:00); (2) antibodies to rBPI₂₁: blood for assessment of antibodies to rBPI₂₁ is drawn at selected study sites at the following times: Day 1 prior to study drug infusion, and Days 15 and 29, if the patient is still in hospital or returns to clinic (actual draw days may vary from Days 15 10-20 and Days 21-29); and (3) cytokines: blood for assessment of cytokines is drawn at selected study sites.

The following safety laboratory panels were assessed at Day 1 prior to test drug infusion, Day 3 (after end of infusion) and Day 8, however, if patient is discharged on or prior to Day 8, assessment was made prior to discharge if possible: (1) hematology panel: hemoglobin, hematocrit, erythrocyte count, leukocyte count and differential, and platelet count; (2) serum chemistry panel: sodium, potassium, chloride, calcium, phosphorous, blood urea nitrogen, creatinine, uric acid, glucose (fasting), CPK, cholesterol, albumin, total protein, AST (SGOT), ALT (SGPT), 25 bilirubin (total), GGT, LDH, and alkaline phosphatase.

The following were recorded for all treated patients through Day 15 and/or Day 29 post-initiation of study drug infusion: (1) adverse events (continued through Day 29); (2) survival status including date and cause(s) of death (continued through Day 29); (3) dates in ICU (continued through Day 29); (4) dates in hospital (continued through Day 29); (5) dates on ventilator (continued through Day 29); (6) 30 dates on dialysis or hemofiltration, specifying method (continued through Day 29);

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(7) concomitant medications, including daily amounts of blood transfused (continued through Day 15 or Day 29); (8) primary surgical procedures performed, for example, including re-operations but excluding procedures like placement of central lines, Swan-Ganz catheters, arterial lines; lumbar punctures, etc. (continued through Day 15); (9) injury severity score (ISS) based on diagnostic evaluations performed during current hospital stay; (10) daily assessment of organ dysfunctions and the presence of infections (continued through Day 15); (11) daily vital signs associated with and including daily maximum and daily minimum temperatures (continued through Day 15); and (12) inspection of infusion site used for study drug administration at least every eight hours, with observations documented in progress notes or the equivalent.

Organ dysfunctions were assessed using the following definitions. The patient was considered to have disseminated intravascular coagulation (DIC) when there were: (1) abnormally low values for platelets (or there was a $> 25\%$ decrease from a previously documented value) and either an elevated prothrombin time or an elevated partial thromboplastin time and clinical evidence of bleeding, or (2) if obtained, a confirmatory test was positive (FDP $> 1:40$ or D-Dimers > 2.0). These abnormalities must have occurred in the absence of medically significant confounding factors such as liver failure, major hematoma, or anticoagulant therapy.

The patient was considered to have acute respiratory distress syndrome (ARDS) when: bilateral pulmonary infiltrates consistent with pulmonary edema were present, and $\text{PaO}_2/\text{FiO}_2 < 200$. These signs must have occurred in the absence of congestive heart failure or primary lung disease such as pulmonary embolus or pneumonia. The Pulmonary Artery Wedge Pressure (PAWP), when measured, must have been < 18 mm Hg.

The patient was considered to have acute renal failure (ARF) when: (1) dialysis or hemofiltration was required (definition used for primary analysis), or (2) serum creatinine became abnormal with an increase of ≥ 2.0 mg/dL in a patient with documented normal baseline creatinine, or (3) serum creatinine was ≥ 3.0 mg/dL in a patient not known to have renal insufficiency, but whose (pretrauma) baseline creatinine was unknown, or (4) serum creatinine was doubled from admission or pre-rBPI₂₁ treatment level in a patient with previous renal insufficiency. These

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findings must not have been prerenal in nature (e.g. associated with dehydration or gastrointestinal bleeding) or due to rhabdomyolysis.

5 Post-surgical hepatobiliary dysfunction (HBD) was evaluated only in patients without primary hepatic disease (e.g., hepatitis or cirrhosis), alcoholism, or biliary disease. The patient was considered to have hepatobiliary dysfunction when: the bilirubin exceeded 3.0 mg/dL, and either the alkaline phosphatase, gamma glutamyl transpeptidase (GGT), alanine aminotransferase (ALT, or SGPT) or aspartate aminotransferase (AST, or SGOT) exceeded twice the upper limit of normal. These findings must have occurred in the absence of confounding disease.

10 Patients were also evaluated for infections in wounds, surgical sites (both superficial and deep incisional sites), organs, anatomical spaces, the bloodstream (bacteremia), the urinary tract, or the respiratory tract (pneumonia).

The physician principal investigators were provided with the definitions of each organ dysfunction and were asked to record at pre-treatment and daily during
15 Days 1-15 (a) whether each organ dysfunction was "Present", "Clinically present", "Not present or clinically present", or "Unknown" according to the definitions provided and (b) all available actual laboratory or clinical data required by the definition, whether or not the definition was met. Investigators were also provided with the definitions of infections and were asked to record (a) whether each type of
20 infection was "Present" or "Not present" at any time during Days 1-15 and (b) if "Present", the actual culture or clinical data required by the definition.

In order to provide a more objective analysis of these endpoints, and upon recommendation of an independent Data Safety Monitoring Board, computer programs were developed prior to the first efficacy interim analysis at 50% accrual
25 to implement the same organ dysfunction and infection definitions using the actual laboratory, clinical and culture data required for each definition. The algorithmic approach defined each organ dysfunction as "Present," "Clinically present," "Not present or clinically present," or "Unknown" at pretreatment and daily during days 1-15. Each organ dysfunction was classified as "Unknown" on any given day if
30 certain minimum assessments required by the definition were not provided on that day. The definition for "Present" on a given day required that all assessments had been made and that each assessment met its respective criterion for the specified

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organ dysfunction. Thus, patients for whom one or more required assessments were missing on a given day could not be classified as having met the definition for "Present" for that organ dysfunction for that day. On days on which assessments were incomplete, the organ dysfunction was classified as "Clinically present" if each of the nonmissing assessments met their respective criteria for "Present" for that organ dysfunction. Therefore, "Clinically present" implies that the organ dysfunction may have been present that day, based on incomplete evidence, and that no contradictory evidence was recorded that day. Since serious ARF was defined as the use of dialysis/hemofiltration on at least one day during Days 1-15, "Clinically present" was not applicable to serious ARF. An organ dysfunction was considered "Not present or clinically present" when none of the definitions for "Present," "Clinically present" or "Unknown" were met for that day. Patients who were classified as having an organ dysfunction "Present" or "Clinically present" pretreatment were required to have satisfied the primary endpoint by another complication in order to have been classified as having met the primary endpoint during Days 1-15.

For infections, the algorithmic approach defined each infection as "Present" or "Not present" during Days 1-15. Each definition for "Present" required that the definition be strictly met on at least one day during Days 1-15 according to the data provided. If the patient did not meet the "Present" definition of infection, they were classified as "Not present" for that infection.

Serious complications were defined as the occurrence of the following serious infections: (1) a deep incisional surgical site infection, (2) an organ or anatomical space infection, (3) a secondary bloodstream infection, (4) a primary bloodstream infection, and (5) pneumonia; or the following serious organ dysfunctions: (1) disseminated intravascular coagulation (DIC) or coagulopathy, (2) acute respiratory distress syndrome (ARDS), (3) acute renal failure (ARF) requiring dialysis or hemofiltration, and (4) hepatobiliary dysfunction (HBD). Patients were counted once as suffering from complications regardless of the number of complications.

Across 19 sites, 1411 patients were screened and 401 patients were randomized into the study groups and received therapy. Among these 401 patients,

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199 received placebo treatment and 202 received rBPI₂₁ treatment. Thirty-one patients (15 placebo, 16 rBPI₂₁) did not receive the complete administration of study drug. Twelve patients (six placebo and six rBPI₂₁) were withdrawn from the study before Day 29. Data from all patients who received any amount of study medication, even if infusion was incomplete, were included in all safety and efficacy analyses, whether or not the patient was withdrawn from the study.

The mean age of the study population was 35 (range: 16-80 years); 80% of the patients were under 45 years of age. The mean dosing weight was 79 kg (range: 45-145 kg). Seventy-seven percent of the patients were male. The traumatic injury source was classified as blunt trauma (50%), penetrating trauma (48%), or both (2%). Of the other trauma related characteristics, the mean TRTS was 10.6 (range: 0-12); the mean GCS was 13.2 (range: 3-15); the mean ISS (version '90) was 23.9 (range: 1-75), the mean number of PRBC units started prior to study drug infusion was 6.4 (range: 0-57), and the mean time from traumatic incident to drug infusion was 9.5 hours (range: 1.3-21.8 hours). There were no notable treatment group differences for age, weight, ethnicity, injury source, TRTS, GCS, and the time to infusion ($p > 0.10$ controlling for site), but the placebo group had a somewhat higher proportion of females ($p = 0.11$, controlling for site). The number of units of packed red blood cells (PRBC) transfused prior to drug infusion was similar between patients randomized to rBPI₂₁ and placebo. The mean ISS was slightly worse ($p = 0.07$ controlling for site) for placebo patients (mean 25.1) than for rBPI₂₁ patients (mean 22.7).

The prespecified primary efficacy analysis focused on the primary endpoint of mortality or serious complication (defined as serious organ dysfunction or serious infection as assessed by the algorithmic approach) occurring at any time after Hour 0 through Day 15. Overall mortality in this study was low (approximately 5-6%). Treatment groups were compared using Cox regression, stratifying by site and unadjusted for covariates. The results were analyzed by computer algorithm using the strict definitions of "Present" and also after incorporating incomplete evidence (leading to classifications of "Present or Clinically present"). Regardless of the algorithmic method, the rate of mortality or serious complication by Day 15 was lower by 7% for rBPI₂₁ patients compared to placebo patients. The Kaplan-

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Meier estimates of event rates for mortality or serious complication using both algorithmic methods are shown below in Table V.

TABLE V

Outcomes and Statistical Calculations		Placebo (N=199)	rBPI ₂₁ (N=202)	p-value
5 "Present" Definition by Algorithm	Actual Event Rate Percentage (#patients with events/ #total patients)	46% (91/199)	39% (78/202)	
	Kaplan-Meier Estimates of Event Rates at 15 days	46%	39%	0.17
10 "Present/ Clinically Present" Definition by Algorithm	Actual Event Rate Percentage (#patients with events/ #total patients)	55% (109/199)	48% (97/202)	
	Kaplan-Meier Estimates of Event Rates at 15 days	55%	48%	0.15

15 Placebo patients were approximately 1.27 times more likely to experience mortality
or serious complication than rBPI₂₁ patients by both algorithmic methods as
measured by the hazard ratio from Cox regression (rBPI₂₁ to placebo hazard ratio
= 0.79; p=0.13 for mortality or serious complication using "Present"; p=0.09 for
20 mortality or serious complication using "Present or Clinically Present", stratified by
center).

As a secondary analysis, the primary analysis was repeated with
adjustment for significant covariates, resulting in the following hazard ratios: rBPI₂₁
to placebo hazard ratio using "Present" = 0.79, p=0.14 (adjusting for age, injury
source, ISS'90 and units PRBC transfused prior to drug infusion); hazard ratio using
25 "Present or Clinically Present" = 0.81, p=0.16 (adjusting for age, ISS'90 and units
PRBC transfused prior to drug infusion). Event incidence for each of the secondary
efficacy measures assessed is shown in Fig. 1. Analysis of these secondary efficacy
measures revealed lower frequencies of the following complications in patients treated
with rBPI₂₁ compared to patients treated with the placebo preparation: any
30 complication, any serious complication, any organ dysfunction, any serious organ

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dysfunction, any infection, any serious infection and pneumonia. Slight reductions were also noted in favor of rBPI₂₁ treatment in the proportion of patients developing disseminated intravascular coagulation or coagulopathy, primary and secondary bloodstream infection and asymptomatic bacteriuria.

5 Adverse events in this severely injured population were frequent in patients treated with either rBPI₂₁ or placebo. There were, however, numerically higher percentages of patients with adverse events in the placebo group compared to the rBPI₂₁ group. A higher percentage of patients were also noted to experience any extremely abnormal post-treatment laboratory result in the placebo treated group
10 compared to the rBPI₂₁ treated group. These data suggest a possible additional beneficial effect.

In summary, this controlled clinical trial evaluating a single dosing regimen has demonstrated a trend in favor of rBPI₂₁ treatment in the primary endpoint of mortality or serious complication through Day 15. Reductions were also
15 noted in the proportion of patients who experienced complications. These results, taken together, are consistent with a beneficial effect for treatment with rBPI₂₁ in patients with hemorrhage due to trauma.

Numerous modifications and variations of the above-described invention are expected to occur to those of skill in the art. Accordingly, only such
20 limitations as appear in the appended claims should be placed thereon.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: XOMA CORPORATION
- (ii) TITLE OF INVENTION: Therapeutic Uses of BPI Protein Products
In Humans With Hemorrhage Due to Trauma
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
 - (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
 - (C) CITY: Chicago
 - (D) STATE: Illinois
 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/652,292
 - (B) FILING DATE: May 23, 1996
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Borun, Michael F.
 - (B) REGISTRATION NUMBER: 25,447
 - (C) REFERENCE/DOCKET NUMBER: 27129/33959 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 312/474-6300
 - (B) TELEFAX: 312/474-0448
 - (C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1813 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 31..1491
- (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) LOCATION: 124..1491
- (ix) FEATURE:

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(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rBPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGGCCTTGA GGTTTTGGCA GCTCTGGAGG ATG AGA GAG AAC ATG GCC AGG GGC	54
Met Arg Glu Asn Met Ala Arg Gly	
-31 -30 -25	
CCT TGC AAC GCG CCG AGA TGG GTG TCC CTG ATG GTG CTC GTC GCC ATA	102
Pro Cys Asn Ala Pro Arg Trp Val Ser Leu Met Val Leu Val Ala Ile	
-20 -15 -10	
GGC ACC GCC GTG ACA GCG GCC GTC AAC CCT GGC GTC GTG GTC AGG ATC	150
Gly Thr Ala Val Thr Ala Ala Val Asn Pro Gly Val Val Val Arg Ile	
-5 1 5	
TCC CAG AAG GGC CTG GAC TAC GCC AGC CAG CAG GGG ACG GCC GCT CTG	198
Ser Gln Lys Gly Leu Asp Tyr Ala Ser Gln Gln Gly Thr Ala Ala Leu	
10 15 20 25	
CAG AAG GAG CTG AAG AGG ATC AAG ATT CCT GAC TAC TCA GAC AGC TTT	246
Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr Ser Asp Ser Phe	
30 35 40	
AAG ATC AAG CAT CTT GGG AAG GGG CAT TAT AGC TTC TAC AGC ATG GAC	294
Lys Ile Lys His Leu Gly Lys Gly His Tyr Ser Phe Tyr Ser Met Asp	
45 50 55	
ATC CGT GAA TTC CAG CTT CCC AGT TCC CAG ATA AGC ATG GTG CCC AAT	342
Ile Arg Glu Phe Gln Leu Pro Ser Ser Gln Ile Ser Met Val Pro Asn	
60 65 70	
GTG GGC CTT AAG TTC TCC ATC AGC AAC GCC AAT ATC AAG ATC AGC GGG	390
Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile Lys Ile Ser Gly	
75 80 85	
AAA TGG AAG GCA CAA AAG AGA TTC TTA AAA ATG AGC GGC AAT TTT GAC	438
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met Ser Gly Asn Phe Asp	
90 95 100 105	
CTG AGC ATA GAA GGC ATG TCC ATT TCG GCT GAT CTG AAG CTG GGC AGT	486
Leu Ser Ile Glu Gly Met Ser Ile Ser Ala Asp Leu Lys Leu Gly Ser	
110 115 120	
AAC CCC ACG TCA GGC AAG CCC ACC ATC ACC TGC TCC AGC TGC AGC AGC	534
Asn Pro Thr Ser Gly Lys Pro Thr Ile Thr Cys Ser Ser Cys Ser Ser	
125 130 135	
CAC ATC AAC AGT GTC CAC GTG CAC ATC TCA AAG AGC AAA GTC GGG TGG	582
His Ile Asn Ser Val His Val His Ile Ser Lys Ser Lys Val Gly Trp	
140 145 150	
CTG ATC CAA CTC TTC CAC AAA AAA ATT GAG TCT GCG CTT CGA AAC AAG	630
Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys	
155 160 165	
ATG AAC AGC CAG GTC TGC GAG AAA GTG ACC AAT TCT GTA TCC TCC AAG	678
Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser Val Ser Ser Lys	
170 175 180 185	
CTG CAA CCT TAT TTC CAG ACT CTG CCA GTA ATG ACC AAA ATA GAT TCT	726
Leu Gln Pro Tyr Phe Gln Thr Leu Pro Val Met Thr Lys Ile Asp Ser	
190 195 200	

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GTG GCT GGA ATC AAC TAT GGT CTG GTG GCA CCT CCA GCA ACC ACG GCT Val Ala Gly Ile Asn Tyr Gly Leu Val Ala Pro Pro Ala Thr Thr Ala 205 210 215	774
GAG ACC CTG GAT GTA CAG ATG AAG GGG GAG TTT TAC AGT GAG AAC CAC Glu Thr Leu Asp Val Gln Met Lys Gly Glu Phe Tyr Ser Glu Asn His 220 225 230	822
CAC AAT CCA CCT CCC TTT GCT CCA CCA GTG ATG GAG TTT CCC GCT GCC His Asn Pro Pro Phe Ala Pro Pro Val Met Glu Phe Pro Ala Ala 235 240 245	870
CAT GAC CGC ATG GTA TAC CTG GGC CTC TCA GAC TAC TTC TTC AAC ACA His Asp Arg Met Val Tyr Leu Gly Leu Ser Asp Tyr Phe Phe Asn Thr 250 255 260 265	918
GCC GGG CTT GTA TAC CAA GAG GCT GGG GTC TTG AAG ATG ACC CTT AGA Ala Gly Leu Val Tyr Gln Glu Ala Gly Val Leu Lys Met Thr Leu Arg 270 275 280	966
GAT GAC ATG ATT CCA AAG GAG TCC AAA TTT CGA CTG ACA ACC AAG TTC Asp Asp Met Ile Pro Lys Glu Ser Lys Phe Arg Leu Thr Lys Phe 285 290 295	1014
TTT GGA ACC TTC CTA CCT GAG GTG GCC AAG AAG TTT CCC AAC ATG AAG Phe Gly Thr Phe Leu Pro Glu Val Ala Lys Lys Phe Pro Asn Met Lys 300 305 310	1062
ATA CAG ATC CAT GTC TCA GCC TCC ACC CCG CCA CAC CTG TCT GTG CAG Ile Gln Ile His Val Ser Ala Ser Thr Pro Pro His Leu Ser Val Gln 315 320 325	1110
CCC ACC GGC CTT ACC TTC TAC CCT GCC GTG GAT GTC CAG GCC TTT GCC Pro Thr Gly Leu Thr Phe Tyr Pro Ala Val Asp Val Gln Ala Phe Ala 330 335 340 345	1158
GTC CTC CCC AAC TCC TCC CTG GCT TCC CTC TTC CTG ATT GGC ATG CAC Val Leu Pro Asn Ser Ser Leu Ala Ser Leu Phe Leu Ile Gly Met His 350 355 360	1206
ACA ACT GGT TCC ATG GAG GTC AGC GCC GAG TCC AAC AGG CTT GTT GGA Thr Thr Gly Ser Met Glu Val Ser Ala Glu Ser Asn Arg Leu Val Gly 365 370 375	1254
GAG CTC AAG CTG GAT AGG CTG CTC CTG GAA CTG AAG CAC TCA AAT ATT Glu Leu Lys Leu Asp Arg Leu Leu Leu Glu Leu Lys His Ser Asn Ile 380 385 390	1302
GGC CCC TTC CCG GTT GAA TTG CTG CAG GAT ATC ATG AAC TAC ATT GTA Gly Pro Phe Pro Val Glu Leu Leu Gln Asp Ile Met Asn Tyr Ile Val 395 400 405	1350
CCC ATT CTT GTG CTG CCC AGG GTT AAC GAG AAA CTA CAG AAA GGC TTC Pro Ile Leu Val Leu Pro Arg Val Asn Glu Lys Leu Gln Lys Gly Phe 410 415 420 425	1398
CCT CTC CCG ACG CCG GCC AGA GTC CAG CTC TAC AAC GTA GTG CTT CAG Pro Leu Pro Thr Pro Ala Arg Val Gln Leu Tyr Asn Val Val Leu Gln 430 435 440	1446
CCT CAC CAG AAC TTC CTG CTG TTC GGT GCA GAC GTT GTC TAT AAA Pro His Gln Asn Phe Leu Leu Phe Gly Ala Asp Val Val Tyr Lys 445 450 455	1491
TGAAGGCACC AGGGGTGCCG GGGGCTGTCA GCCGCACCTG TTCCTGATGG GCTGTGGGGC	1551
ACCGGCTGCC TTTCCCCAGG GAATCCTCTC CAGATCTTAA CCAAGAGCCC CTTGCAAACT	1611

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TCTTCGACTC AGATTGAGAA ATGATCTAAA CACGAGGAAA CATTATTCAT TGGAAAAGTG 1671
 CATGGTGTGT ATTTTAGGGA TTATGAGCTT CTTTCAAGGG CTAAGGCTGC AGAGATATTT 1731
 CCTCCAGGAA TCCTGTTTCA ATGTGAACCA AGAAATTTC CATTGTGCTT CATGAAAAAA 1791
 AACITCTGGT TTTTTCATG TG 1813

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 487 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val
 -31 -30 -25 -20
 Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
 -15 -10 -5 1
 Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
 5 10 15
 Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
 20 25 30
 Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
 35 40 45
 His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
 50 55 60 65
 Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
 70 75 80
 Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
 85 90 95
 Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
 100 105 110
 Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
 115 120 125
 Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
 130 135 140 145
 Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
 150 155 160
 Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
 165 170 175
 Val Thr Asn Ser Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
 180 185 190
 Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu
 195 200 205
 Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
 210 215 220 225

- 30 -

Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro
 230 235 240
 Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly
 245 250 255
 Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala
 260 265 270
 Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser
 275 280 285
 Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val
 290 295 300 305
 Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser
 310 315 320
 Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro
 325 330 335
 Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala
 340 345 350
 Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser
 355 360 365
 Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu
 370 375 380 385
 Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu
 390 395 400
 Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val
 405 410 415
 Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val
 420 425 430
 Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe
 435 440 445
 Gly Ala Asp Val Val Tyr Lys
 450 455

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of treating a human suffering from hemorrhage due to trauma comprising the step of administering a therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to said human.
- 5 2. The method of claim 1 wherein the BPI protein product is an amino-terminal fragment of BPI protein having a molecular weight of about 21 kD to 25 kD.
3. The method of claim 1 wherein the BPI protein product is rBPI₂₃ or a dimeric form thereof.
4. The method of claim 1 wherein the BPI protein product is rBPI₂₁.
- 10 5. The method of any one of claims 1 to 4, further comprising the administration of at least one unit of blood or packed red blood cells.
6. Use of a BPI protein product in the manufacture of a medicament for the treatment of humans suffering from hemorrhage due to trauma.
7. Use according to claim 6, wherein the BPI protein product is selected from the
15 group consisting of amino-terminal fragments of BPI protein having a molecular weight of about 21 kD to 25 kD, rBPI₂₃ or dimeric forms thereof, and rBPI₂₁.
8. Method of treating a pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma comprising the step of administering a therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to said
20 human.



9. A method of claim 8, wherein the BPI protein product is an amino-terminal fragment of BPI protein having a molecular weight of about 21 kD to 25 kD.
10. A method of claim 8, wherein the BPI protein product is rBPI₂₃ or a dimeric form thereof.
- 5 11. A method of claim 8, wherein the BPI protein product is rBPI₂₁.
12. A method of any one of claims 8 to 11, wherein said pulmonary dysfunction complication is pneumonia.
13. A method of any one of claims 8 to 11, wherein the human suffering from hemorrhage due to trauma is additionally administered at least one unit of blood or
10 packed red blood cells.
14. A method of any one of claims 8 to 12, wherein the BPI protein product is administered to said human before development of the pulmonary dysfunction complication.
15. Use of a BPI protein product in the manufacture of a medicament for treating a
15 pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma.
16. Use according to claim 15, wherein the BPI protein product is selected from the group consisting of amino-terminal fragments of BPI protein having a molecular weight of about 21 kD to 25 kD, rBPI₂₃ or dimeric forms thereof, and rBPI₂₁.
- 20 17. Use according to claim 14 or claim 15, wherein said pulmonary dysfunction complication is pneumonia.



18. Use according to any one of claim 16 to 17, wherein the human suffering from hemorrhage due to trauma is additionally administered at least one unit of blood or packed red blood cells.

19. Use according to any one of claims 15 to 18, wherein the medicament is intended
5 to be administered before development of the pulmonary dysfunction complication.

20. Method of treating a human suffering from hemorrhage due to trauma comprising the step of administering a therapeutically effective amount of a BPI protein product, substantially as herein described with reference to any one of the examples.

21. Use of a BPI protein product in the manufacture of a medicament for the treatment
10 of humans suffering from hemorrhage due to trauma, substantially as herein described with reference to any one of the examples.

22. Method of treating pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma comprising the step of administering a BPI protein product, substantially as herein described with reference to any one of the examples.

23. Use of BPI protein product in the manufacture of a medicament for treating a
15 pulmonary dysfunction complication in a human suffering from hemorrhage due to trauma, substantially as herein described with reference to any one of the examples.

DATED this 5TH day of June 2001

XOMA CORPORATION

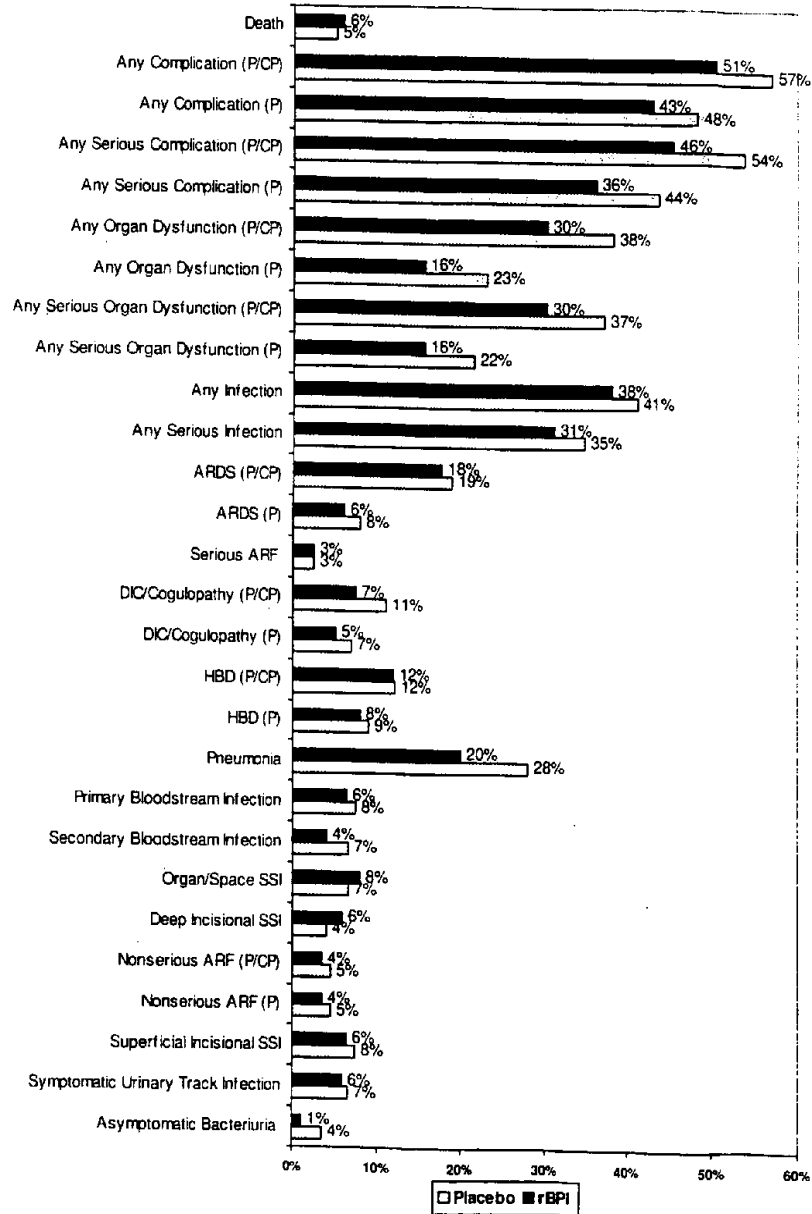
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Attorney: IVAN A. RAJKOVIC
Fellow Institute of Patent Attorneys of Australia
of BALDWIN SHELSTON WATERS



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FIG. 1



SUBSTITUTE SHEET (RULE 26)