

Use of serine protease inhibitors in the treatment of neutropenia

Field of the Invention

The invention relates to therapeutic compounds which are inhibitors of serine proteases, to pharmaceutical compositions thereof and to their use in the treatment of the human or animal body. More specifically, the present invention relates to a method for the treatment of neutropenia comprising the administration to a subject in need thereof of a therapeutically effective amount of a serine protease inhibitor. The invention also comprises prevention of apoptosis of myeloid cells (1) during and after transfection of bone marrow cells performed for gene therapy, (2) during blood stem cell mobilization performed for reconstitution of hematopoiesis and (3) during infusion of cells of the myeloid lineage for reconstitution of hematopoiesis for gene therapy or for treatment of neutropenia by infusion of neutrophils.

Background of the Invention

The invention relates to the use of compounds which are inhibitors of serine proteases. Proteases or proteolytic enzymes are essential in organisms, from bacteria and viruses to mammals. Proteases digest and degrade proteins by hydrolyzing peptide bonds. Serine proteases (EC. 3.4.21) have common features in the active site, primarily an active serine residue. There are two main types of serine proteases; the chymotrypsin/trypsin/elastase-like and subtilisin-like, which have an identical spatial arrangement of catalytic His, Asp, and Ser but in quite different protein scaffolds. However, over twenty families (S1-S27) of serine proteases have been identified that are grouped into 6 clans on the basis of structural similarity and other functional evidence, SA, SB, SC, SE, SF & SG. Family of chymotrypsin/trypsin/elastase-like serine proteases have been subdivided into two classes. The "large" class (ca 230 residues) includes mostly mammalian enzymes such as trypsin, chymotrypsin, elastase, kallikrein, and thrombin. The "small" class (ca 190 residues) includes the bacterial enzymes.

The catalytic His, Asp and Ser are flanked by substrate amino acid side chain residue binding pockets termed S1', S2', S3' etc on the C-terminal or 'prime' side of the substrate and S1, S2, S3 etc on the N-terminal side. This nomenclature is as described in Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding,

Alan Fersht, 1999 (W.H. Freeman and Company) pages 40-43 and Brik et al, *Org. Biomol. Chem.*, 2003, 1, 5-14. The chymotrypsin/trypsin/elastase-like serine proteases can also be further subdivided by the residues present in the S1 pocket as described in *Introduction to Protein Structure*, Carl Branden and John Tooze, 1991 (Garland Publishing Inc) pages 231-241. The subdivisions are chymotrypsin-like (Gly-226, Ser-189 and Gly-216 in S1 pocket), trypsin-like (Gly-226, Asp-189 and Gly-216 in S1) and elastase-like (Val-226 and Thr-216 in S1) where the residues numbering is taken from the standard chymotrypsin numbering. The trypsin-like serine proteases prefer substrates which place either Lys or Arg in the S1 pocket.

The serine proteases have a common catalytic mechanism characterized by a particularly reactive Ser residue at position 195 using the chymotrypsin numbering system. Examples of serine proteases include trypsin, tryptase, chymotrypsin, elastase, thrombin, plasmin, kallikrein, Complement C1, acrosomal protease, lysosomal protease, cocoonase, α -lytic protease, protease A, protease B, serine carboxypeptidase π , subtilisin, urokinase (uPA), Factor VIIIa, Factor IXa, and Factor Xa. The serine proteases have been investigated extensively for many years and are a major focus of research as a drug target due to their role in regulating a wide variety of physiological processes.

Processes involving serine proteases include coagulation, fibrinolysis, fertilization, development, malignancy, neuromuscular patterning and inflammation. It is well known that these compounds inhibit a variety of circulating proteases as well as proteases that are activated or released in tissue. It is also known that serine protease inhibitors inhibit critical cellular processes, such as adhesion, migration, free radical production and apoptosis. In addition, animal experiments indicate that intravenously administered serine protease inhibitors, variants or cells expressing serine protease inhibitors, provide protection against tissue damage.

Serine protease inhibitors have also been predicted to have potential beneficial uses in the treatment of disease in a wide variety of clinical areas such as oncology, hematology, neurology, pulmonary medicine, immunology, inflammation and infectious disease. Serine protease inhibitors may also be beneficial in the treatment of thrombotic diseases, asthma, emphysema, cirrhosis, arthritis, carcinoma, melanoma, restenosis, atheroma, trauma, shock and reperfusion injury. A useful review is found in *Expert Opin. Ther. Patents* (2002), 12(8). Serine protease inhibitors are disclosed in US published patent applications US

2003/0100089 and 2004/0180371 and in US patents 6,784,182, 6,656,911, 6,656,910, 6,608,175, 6,534,495 and 6,472,393.

Leukopenia refers to a decrease in the total leukocyte count below about 4.0×10^9 cells/L. Usually, the reduction is a result of a decrease in the number of polymorphonuclear neutrophils (PMN) (neutropenia), their numbers being usually less than 2.0×10^9 cells/L and frequently below 1.0×10^9 cells/L. Neutropenia may result from viral infections (e.g. influenza, measles, hepatitis virus, chickenpox, dengue and yellow fever, HIV) or from overwhelming bacterial infections including miliary tuberculosis and septicemia. Furthermore, neutropenia develops due to irradiation or treatment with drugs used e.g. in chemotherapy of malignant diseases or vasculitis and autoimmune diseases. Examples for drug-induced neutropenia are sulfonamides, antithyroid drugs, antihistamines, antimicrobial agents, phenothiazines and various analgesics, sedatives and anti-inflammatory agents or various toxic chemicals. Induction of cell death by infectious agents, drugs and toxic chemicals or antibodies may affect neutrophils and/or their precursor cells in the bone marrow. Antibodies to cells of the myeloid lineage are seen in immune mediated diseases such as systemic lupus erythematoses or juvenile rheumatoid arthritis. Last but not least various forms of congenital neutropenia have been described. Neutropenia results not only from damage of PMN in the circulation, but also from damage of stem cells and mitotic cells in the bone marrow by infectious agents, drugs, irradiation and toxic chemicals or due to slowing of cell divisions, blockade of DNA strand duplication, RNA formation or disruption of the microtubules of the mitotic spindle.

Neutropenia e.g. due to chemotherapy for hematologic malignancies, solid tumors or carcinomas leads to an impaired host response with significant morbidity and mortality due to infections. For example chemotherapy of early breast cancer with cyclophosphamide, methotrexate and fluorouracil results in neutropenic events in 30% of the patients with sepsis with requirement for delay of further anti-cancer treatment or dose reduction. Dose reductions of 20-30% have been associated with lower complete response rates and shortened survival in patients with lymphoma or with inferior relapse – free survival. Despite of improvements in antibacterial therapy for neutropenic sepsis, each year approximately 5% of patients receiving myelotoxic chemotherapy die due to infection related complications.

In-vitro handling of neutrophils and their precursor cells e.g. for gene therapy or for preparation of infusions of neutrophils is associated with an increase of cell death due to induction of apoptosis of myeloid cells.

Present agents used for the treatment of neutropenia include G-CSF, GM-CSF and G-CSF conjugated to polyethylene glycol as pegulated G-CSF. Despite the availability and considerable efficacy of the above approved agents in reducing the risk of neutropenia ~~and~~ its complications remain significant issues in oncology. Rarely rupture of the spleen but more frequently increase of the spleen volume, disturbances of gas exchange in the lung and single cases of acute injury stroke and myocardial infarction have been observed in healthy donors receiving G-CSF for harvesting peripheral blood stem cells. The evidences that G-CSF causes myelodysplastic syndromes and acute myeloid leukemia are less clear and need to be analyzed in further prospective long-term studies.

Although these approaches have shown promise, there is a need of improved therapeutic, prophylactic or diagnostic approaches for the treatment of neutropenia. The present invention provides an improved and reliable method for the treatment, diagnosis or prophylaxis of neutropenia comprising the administration to a subject in need thereof of a therapeutically effective amount of a Serine protease inhibitor.

These and other objects as will be apparent from the foregoing have been achieved by the present invention.

Summary of the Invention

The present invention concerns a method for the treatment or prevention of patients suffering from neutropenia comprising the administration to said patients in need thereof of a therapeutically effective amount of serine protease inhibitors. Preferably the serine protease inhibitors is a Kallikrein inhibitor and preferably said Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof. The most preferably said Kallikrein inhibitor is selected among hK2, hK4, hK11, hK5, hK14 inhibitors or mixtures thereof. Even more preferably said Kallikrein inhibitor is an hK2 inhibitor. Preferably the serine protease inhibitors are selected from the

group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

Also disclosed are serine protease inhibitors for use in a method of treating or preventing neutropenia in patients which develops due to infections, septicemia, chemotherapy, irradiation, toxic chemicals or as side effects of any medication. Preferably, the number and / or activation state of neutrophils is impaired. Said serine protease inhibitors are also for use in a method of treating or preventing skin ulcers in diabetes patients in which neutrophils undergo cell death, or skin ulcers developing in patients with peripheral arterial disease associated with hypoxic conditions in the skin and neutrophil dysfunction and apoptosis.

Also said serine protease inhibitors are for use in a method of treating or preventing irradiation induced damage of myeloid cells as occurs in the course of treatment of malignancy, accidents in nuclear plants or use of nuclear weapons. Preferably said serine protease inhibitors is a Kallikrein inhibitor and preferably said Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof. Preferably said serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

Further disclosed are serine protease inhibitors for use in the *in-vitro* preparation of neutrophils and their bone marrow precursors

- to perform molecular manipulations for gene therapy prior to infusion of myeloid cells to patients with neutropenia or genetic disorders of the myeloid system,
- or to use neutrophils and their bone marrow precursors for infusion to patients with neutropenia or dysfunction of neutrophils.

Preferably said serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

The invention further provides a method for the prevention of apoptosis of myeloid cells of patients, comprising the administration to said patients in need thereof of a therapeutically effective amount of serine protease inhibitors:

- (1) during and after transfection of bone marrow cells performed for gene therapy,
- (2) during blood stem cell mobilization performed for reconstitution of hematopoiesis and/or

(3) during infusion of cells of the myeloid lineage for reconstitution of hematopoiesis for gene therapy or for treatment of neutropenia by infusion of neutrophils.

Preferably said serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

The invention also provides a kit for the diagnosis, prognosis, prophylaxis or treatment of neutropenia in a mammal, characterized in that said kit comprises serine protease inhibitors, optionally with reagents and/or instructions for use. Preferably said serine protease inhibitors comprise a detectable label or can bind to a detectable label to form a detectable complex. Also preferably said serine protease inhibitors is a Kallikrein inhibitor and preferably said Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof. Preferably said serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing detailed description, which proceeds with reference to the following illustrative drawings, and the attendant claims.

Brief description of the figures

Figure 1: shows Annexin-V staining of neutrophils and T-cells upon incubation with protease inhibitors MDPK67b and MDOKG9.

(a) Annexin-V staining of neutrophils and T-cells upon incubation with MDPK67b. Cells were incubated for 24 or 48 hours with MDPK67b at concentrations ranging from 6 μ M to 60 μ M, as indicated, or PBS as control. Apoptosis was assessed by Annexin V staining and FACS analysis. Indicated leukocyte populations were gated based on their appearance in a forward scatter/ sideward scatter FACS dot plot (neutrophils) or by positive staining for CD3 (T cells).

(b) Annexin-V staining of neutrophils upon incubation with MDPK67b or MDOKG9 (OKDG9).

Neutrophils were incubated for 18 hours with MDPK67b or MDOKG9 concentrations ranging from 60 μ M (dilution 1) to 60 pM (dilution 7) as indicated. Apoptosis was assessed as outlined above.

Figure 2: shows comparison of various cell culture conditions through Annexin-V staining of MDPK67b treated neutrophils.

Neutrophils were cultured with the indicated concentrations of MDPK67b. PBS without MDPK67b served as a control. Neutrophils were plated (100 μ l/well) either at 5×10^6 /ml (high density) or 3×10^5 /ml (low density) and neutrophil apoptosis was assessed by AnnexinV staining and FACS analysis. Culturing of 5×10^6 /ml neutrophils in serum free medium (X-Vivo 15) instead of RPMI10% FCS was assessed in parallel.

Figure 3: shows reversion of MDPK67b mediated neutrophil protection by tyrosine kinase inhibitors.

(a) Effect of MDPK67b on CD16 and CD11b levels of cultured neutrophils. Neutrophils were cultured with the indicated concentrations of MDPK67b and percentage of neutrophils expressing high levels of CD16 or CD11b was assessed by FACS. Representative FACS plots are shown.

(b) Reversion of effect of MDPK67b on CD16 and CD11b neutrophils levels by PP2.

Neutrophils were cultured with the indicated concentrations of MDPK67b in presence or absence of the Src tyrosine kinase inhibitor PP2 (final concentration 10 μ M). Apoptosis and relative frequencies of CD11b and CD16 high expressing neutrophils were measured by FACS analysis.

Figure 4: shows effect of G-CSF on neutrophil in vitro apoptosis.

Neutrophils were cultured with the indicated concentrations of G-CSF and neutrophil (a) Apoptosis and (b) down-regulation of CD16 expression were analyzed by FACS. (c) Neutrophils were cultured with MDPK67b (0.6 μ M) and titrated amounts of G-CSF (concentrations as indicated). Neutrophils cultured in medium and PBS (without MDPK67b) served as a control.

Figure 5: shows Annexin-V and CD16 staining of neutrophils treated with MDPK67b and Etoposid.

- (a) Annexin-V staining of neutrophils treated with MDPK67b and Etoposid. Cells were incubated during 18 hours with MDPK67b (6 μ M) plus Etoposid (125 μ g/ml), Etoposid alone or PBS. Apoptosis was assessed by Annexin V staining and FACS analysis. Relevant leukocyte populations were gated based on their appearance in a forward scatter or sideward scatter FACS dot plot.
- (b) Annexin-V staining of neutrophils treated with low MDPK67b and increasing Etoposid concentrations. Cells were incubated for 18 hours with MDPK67b (0.06 μ M) alone or MDPK67b (0.06 μ M) plus increasing concentrations of Etoposid (in μ g/ml) as indicated or PBS. Apoptosis was assessed by Annexin V staining and FACS analysis was performed as mentioned above.
- (c) CD16 staining of neutrophils treated with MDPK67b and Etoposid. Cells were incubated for 18 hours with MDPK67b (0.06 μ M) alone or MDPK67b (0.06 μ M) plus increasing concentrations of Etoposid (in μ g/ml) as indicated or PBS. Percentages of CD16 high expressing neutrophils were assessed by FACS analysis.

Detailed Description of the Invention

Some of the serine proteases of the chymotrypsin superfamily, including t-PA, plasmin, u-PA and the proteases of the blood coagulation cascade are large molecules that contain, in addition to the serine protease catalytic domain, other structural domains responsible in part for regulation of their activity (Barrett, 1986; Gerard et al, 1986; Blasi et al., 1986).

Among important serine proteases are trypsin-like enzymes, such as trypsin, tryptase, thrombin, kallikrein, and factor Xa. The serine protease targets are associated with processes such as blood clotting; complement mediated lysis, the immune response, inflammation, pain sensing, glomerulonephritis, pancreatitis, cancer, regulating fertilization, bacterial infection and viral maturation. By inhibiting serine proteases which have high specificity for a particular target, one can inhibit in vivo numerous biological processes, which may have dramatic effects on a host.

Serine proteinase inhibitors (serpins) comprise a diverse group of proteins that form a superfamily already including more than 100 members, from such diverse organisms as viruses, plants and humans. Serpins have evolved over 500 million years and diverged phylogenetically into proteins with inhibitory function and non-inhibitory function (Hunt and Dayhoff, 1980). Non-inhibitory serpins such as ovalbumin lack protease inhibitory

activity (Remold-O'Donnell, 1993). The primary function of serpin family members appears to be the neutralization of overexpressed serine proteinase activity (Potempa et al., 1994). Serpins play a role in extracellular matrix remodeling, modulation of inflammatory response and cell migration (Potempa et al., 1994).

Serine protease inhibitors are divided into the following families: the bovine pancreatic trypsin inhibitor (Kunitz) family, also known as basic protease inhibitor (Ketcham et al., 1978); the Kazal family; the *Streptomyces subtilisin* inhibitor family; the serpin family; the soybean trypsin inhibitor (Kunitz) family; the potato inhibitor family; and the Bowman-Birk family (Laskowski et al., 1980; Read et al., 1986; Laskowski et al., 1987). Serine protease inhibitors belonging to the serpin family include the plasminogen activator inhibitors PAI-1, PAI-2 and PAI-3, C1 esterase inhibitor, alpha-2-antiplasmin, contrapsin, alpha-1-antitrypsin, antithrombin III, protease nexin I, alpha-1-antichymotrypsin, protein C inhibitor, heparin cofactor II and growth hormone regulated protein (Carrelletal., 1987; Sommeretal., 1987; Suzuki et al., 1987; Stump et al., 1986).

Many of the serine protease inhibitors have a broad specificity and are able to inhibit both the chymotrypsin superfamily of proteases, including the blood coagulation serine proteases, and the *Streptomyces subtilisin* superfamily of serine proteases (Laskowski et al., 1980). The inhibition of serine proteases by serpins has been reviewed in Travis et al. (1983); Carrelletal. (1985); and Sprengers et al. (1987). Crystallographic data are available for a number of intact inhibitors including members of the BPTI, Kazal, SSI, soybean trypsin and potato inhibitor families, and for a cleaved form of the serpin alpha-1-antitrypsin (Read et al., 1986). Despite the fact that these serine protease inhibitors are proteins of diverse size and sequence, the intact inhibitors studied to date all have in common a characteristic loop, termed the reactive site loop, extending from the surface of the molecule that contains the recognition sequence for the active site of the cognate serine protease (Levin et al., 1983). The structural similarity of the loops in the different serine protease inhibitors is remarkable (Papamokos et al., 1982). The specificity of each inhibitor is thought to be determined primarily by the identity of the amino acid that is immediately amino-terminal to the site of potential cleavage of the inhibitor by the serine protease. This amino acid, known as the Pi site residue, is thought to form an acyl bond with the serine in the active site of the serine protease (Laskowski et al., 1980). Whether or not a serpin possesses inhibitory function depends strongly on the consensus sequence located in the hinge region of the reactive site loop near the carboxy-terminus of the

coding region. Outside of the reactive site loop, the serine protease inhibitors of different families are generally unrelated structurally, although the Kazal family and Streptomyces subtilisin family of inhibitors display some structural and sequence similarity.

As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

"A" or "an" means "at least one" or "one or more."

The term "comprise" is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

As used herein, the terms "protein", "polypeptide", "polypeptidic", "peptide" and "peptidic" or "peptidic chain" are used interchangeably herein to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

"Amino acid residue" means any amino acid residue known to those skilled in the art. This encompasses naturally occurring amino acids (including for instance, using the three-letter code, Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val), as well as rare and/or synthetic amino acids and derivatives thereof (including for instance Aad, Abu, Acp, Ahe, Aib, Apm, Dbu, Des, Dpm, Hyl, McLys, McVal, Nva, and the like).

Said amino acid residue or derivative thereof can be any isomer, especially any chiral isomer, e.g. the L- or D- isoform.

By amino acid derivative, we hereby mean any amino acid derivative as known in the art. For instance, amino acid derivatives include residues derivable from natural amino acids bearing additional side chains, e.g. alkyl side chains, and/or heteroatom substitutions.

"Fragments" refer to sequences sharing at least 40% amino acids in length with the respective sequence of the substrate active site. These sequences can be used as long as they exhibit the same properties as the native sequence from which they derive. Preferably these sequences share more than 70%, preferably more than 80%, even more preferably more than 90%, in

particular more than 95% amino acids in length with the respective sequence the substrate active site.

The present invention also includes variants of the substrate active site sequence. The term “variants” refer to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide that is amino acid sequences that vary from the native sequence by conservative amino acid substitutions, whereby one or more amino acids are substituted by another with same characteristics and conformational roles. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence. Conservative amino acid substitutions are herein defined as exchanges within one of the following five groups:

- I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly
- II. Polar, positively charged residues: His, Arg, Lys
- III. Polar, negatively charged residues: and their amides: Asp, Asn, Glu, Gln
- IV. Large, aromatic residues: Phe, Tyr, Trp
- V. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys.

The term “kallikrein” relates to glandular or tissue kallikreins. Glandular or tissue kallikreins are a sub-family of serine proteases, with a high degree of substrate specificity and diverse expression in various tissues and biological fluids. The term "kallikrein" appeared in the literature for the first time in the 1930s, when large amounts of protease enzymes were found in pancreas isolates (pancreas is "Kallikreas" in Greek) (Kraut *et al.* 1930, Werle 1934).

Nowadays kallikrein enzymes are divided into two groups, plasma and tissue kallikreins, which differ significantly in their molecular weight, substrate specificity, immunological characteristics, gene structure, and type of the kinin released.

Kallikreins comprise a family of 15 homologous single chain, secreted serine endopeptidases of ~25–30 kDa, with orthologues present in species from at least six mammalian orders.

These kallikreins are hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14 and hK15. Preferably kallikreins are hK2, hK4, hK11 and hK14.

“Antibody”, as used herein, refers to a class of plasmaproteins produced by the B-cells of the immune system after stimulation by an antigen. Mammal (i.e. Human) antibodies are immunoglobulins of the Ig G, M, A, E or D class. The term “antibody” as used for the purposes of this invention includes, but is not limited to, polyclonal, monoclonal, chimeric,

humanized, human, internalizing, neutralizing, anti-idiotypic antibodies, immunologically-active fragments or derivatives thereof, recombinant proteins having immunologically-activity, and immunoconjugates which bind a kallikrein or a membrane anchored serine protease.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

“Disease”, as used herein, refers to a pathological condition of a part, organ, or system of an organism resulting from various causes, such as infection, genetic defect, or environmental stress, and characterized by an identifiable group of signs or symptoms.

“Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, monkeys etc. Preferably, the mammal is human.

“Treatment” refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Hence, the mammal to be treated herein may have been diagnosed as having the disorder or may be predisposed or susceptible to the disorder.

The term "subject" refers to patients of human or other mammal and includes any individual it is desired to examine or treat using the methods according to the present invention. However, it will be understood that “patient” does not automatically imply that symptoms or diseases are present.

The phrase “pharmaceutically acceptable” refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

As used herein, the term "protease" refers to a class of enzymes which recognizes a molecule and cleaves an activation sequence in the molecule. The protease can be an endopeptidase which cleaves internal peptide bonds. Alternatively, the protease can be an exopeptidase which hydrolyzes the peptide bonds from the N-terminal end or the C-terminal end of the

polypeptide or protein molecule. The protease folds into a conformation to form a catalytic site which receives and cleaves the activation sequence.

“Inhibitors” refer to a polypeptide, or a chemical compound, that specifically inhibit the function of a kallikrein or serine protease by, preferably, binding to said kallikrein or serine protease.

“Reactive Serpin Loop” or “Reactive Site Loop” or RSL refers to an exposed flexible reactive-site loop found in serpin and which is implicated in the interaction with the putative target protease. From the residue on the amino acid side of the scissile bond, and moving away from the bond, residues are conventionally called P1, P2, P3, *etc.* Residues that follow the scissile bond are called P1', P2', P3', *etc.* Usually, the RSL is composed of 6 to 12 amino acid residues.

“Serine protease” or serpin according to the invention can be selected from the group comprising the α -1antichymotrypsin (ACT), protein C inhibitor (PCI), α -1antitrypsinase (AAT), human α -1antitrypsin-related protein precursor (ATR), α -2-plasmin inhibitor (AAP), human anti-thrombin-III precursor (ATIII), protease inhibitor 10 (PI10), human collagen-binding protein 2 precursor (CBP2), protease inhibitor 7 (PI7), protease inhibitor leuserpin 2 (HLS2), human plasma protease C1 inhibitor (C1 INH), monocyte/neutrophil elastase inhibitor (M/NEI), plasminogen activator inhibitor-3 (PAI3), protease inhibitor 4 (PI4), protease inhibitor 5 (PI5), protease inhibitor 12 (PI12), human plasminogen activator inhibitor-1 precursor endothelial (PAI-1), human plasminogen activator inhibitor-2 placental (PAI2), human pigment epithelium-derived factor precursor (PEDF), protease inhibitor 6 (PI6), protease inhibitor 8 (PI8), protease inhibitor 9 (PI9), human squamous cell carcinoma antigen 1 (SCCA-1), human squamous cell carcinoma antigen 2 (SCCA-2), T4-binding globulin (TBG), Megsin, and protease inhibitor 14 (PI14), fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

Since most of these serpins have different names, Applicant includes below a table summarizing their specifications:

Table I

Serpin	Accession Number	RSL sequence
PI or AAT, A1AT_HUMAN ALPHA-1-ANTITRYPSIN PRECURSOR (ALPHA-1 PROTEASE INHIBITOR) (ALPHA-1- ANTIPROTEINASE)	sp P01009	GTEAAGAMFLEAIPMSIPPE
PIL or ATR, A1AU_HUMAN ALPHA-1-ANTITRYPSIN-RELATED PROTEIN PRECURSOR	sp P20848	GTEATGAPHLEEKAWSKYQT
PLI OR AAP, A2AP_HUMAN ALPHA-2-ANTIPLASMIN PRECURSOR (ALPHA-2-PLASMIN INHIBITOR) (ALPHA-2-PI) (ALPHA-2-AP)	sp P08697	GVEAAAAATSIAMSRMSLSFF
AACT, AACT_HUMAN ALPHA-1-ANTICHYMOTRYPSIN PRECURSOR (ACT)	sp P01011	GTEASAATAVKITLLSALVE
AT3, ANT3_HUMAN ANTITHROMBIN-III PRECURSOR (ATIII)	sp P01008	GSEAAASTAVVIAGRSINPN
PI10, BOMA_HUMAN BOMAPIN (PROTEASE INHIBITOR 10)	sp P48595	GTEAAAGSGSEIDIRIVPS
CBP2, CBP2_HUMAN COLLAGEN-BINDING PROTEIN 2 PRECURSOR (COLLIGIN 2)	sp P50454	GNPFDQDIYGREELRSPKLF
PI7 or PN1, GDN_HUMAN GLIA DERIVED NEXIN PRECURSOR (GDN) (PROTEASE NEXIN I) (PN-1) (PROTEASE INHIBITOR 7)	sp P07093	GTKASAATTAILIARSSPPW
HCF2, HEP2_HUMAN HEPARIN COFACTOR II PRECURSOR (HC-II) (PROTEASE INHIBITOR LEUSERPIN 2) (HLS2)	sp P05546	GTQATTVTTVGFMPPLSTQVR
C1NH or C1IN, IC1_HUMAN PLASMA PROTEASE C1 INHIBITOR PRECURSOR (C1 INH)	sp P05155	GVEAAAAAISVARTLLVFE
ELANH2 or PI2, ILEU_HUMAN LEUKOCYTE ELASTASE INHIBITOR (LEI) (MONOCYTE/NEUTROPHIL ELASTASE INHIBITOR) (M/NEI) (EI)	sp P30740	GTEAAAATAGIATFCMLMPE
PCI or PLANH3 or PROCI, IPSP_HUMAN PLASMA SERINE PROTEASE INHIBITOR PRECURSOR (PCI) (PROTEIN C INHIBITOR) (PLASMINOGEN ACTIVATOR INHIBITOR-3) (PAI3)	sp P05154	GTRAAAATGTIFTFRSARLN
PI4 or KST, KAIN_HUMAN KALLISTATIN PRECURSOR (KALLIKREIN INHIBITOR) (PROTEASE INHIBITOR 4)	sp P29622	GTEAAAATTFAIKFFSAQTN
PI5, MASP_HUMAN MASPIN PRECURSOR (PROTEASE INHIBITOR 5)	sp P36952	GGDSIEVPGARILQHKDELN
PI12, NEUS_HUMAN NEUROSERPIN PRECURSOR (PROTEASE INHIBITOR 12)	sp Q99574	GSEAAAVSGMIAISRMAVLY
PAI1 or PLANH1, sp P05121 PAI1_HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-1 PRECURSOR, ENDOTHELIAL (PAI-1)	sp P05121	GTVASSSTAVIVSARMAPEE
PAI2 or PLANH2, PAI2_HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-2, PLACENTAL (PAI-2) (MONOCYTE ARG- SERPIN) (UROKINASE INHIBITOR)	sp P05120	GTEAAAGTGGVMTGRGTGHGG
PEDF, PEDF_HUMAN PIGMENT EPITHELIUM-DERIVED FACTOR PRECURSOR (PEDF) (EPC-1)	sp P36955	GAGTTPSPGLQPAHLTFPLD
PI6 or PTI, PTI6_HUMAN PLACENTAL THROMBIN INHIBITOR (CYTOPLASMIC ANTIPROTEINASE) (CAP) (PROTEASE INHIBITOR 6)	sp P35237	GTEAAAATAAIMMRCARFV
PI8, PTI8_HUMAN CYTOPLASMIC ANTIPROTEINASE 2 (CAP2) (CAP-2) (PROTEASE INHIBITOR 8)	sp P50452	GTEAAAATAVVNRNSRCSRME
PI9, PTI9_HUMAN CYTOPLASMIC ANTIPROTEINASE 3 (CAP3) (CAP-3) (PROTEASE INHIBITOR 9)	sp P50453	GTEAAAASSCFVVAECCMES
SCCA1, SCC1_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1) (PROTEIN T4-A)	sp P29508	GAEAAAATAVVGFGSSPAST
SCCA2, SCC2_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2) (LEUPIN)	sp P48594	GVEAAAATAVVVVVELSSPST
TBG, THBG_HUMAN THYROXINE-BINDING GLOBULIN PRECURSOR (T4-BINDING GLOBULIN)	sp P05543	GTEAAAVPEVELSDQPENTF
MEGSIN	gi 4505149 ref NP_003775.1	GTEATAATGSNIVEKQLPQS
PI14, pancpin, TSA2004	gi 3724282 dbj BAA33766.1	GSEAAATSTGIHIPVIMSLAQ

Advantageously, the serine protease inhibitor of the invention may be a serine protease trypsin-like enzyme and preferably a Kallikrein inhibitor. Kallikrein inhibitors of the invention are selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14 or hK15 inhibitors. Preferably kallikreins inhibitors are selected among hK2, hK4, hK11, hK5 and hK14 inhibitors. More preferably, the kallikrein inhibitor is an hK2 inhibitor.

Encompassed by the present invention are recombinant inhibitor proteins of a Kallikrein, comprising a serpin sequence wherein the Reactive Serpin Loop P6-P6' of said serpin sequence comprises at least one substrate active site sequence specific for said Kallikrein, biologically active fragments thereof, a molecular chimera thereof, a combination thereof and/or variants thereof. Said at least one substrate active site sequence specific for said Kallikrein is a substrate peptide selected by Kallikrein using a phage-displayed random pentapeptide library as disclosed in International Patent Application PCT/IB2004/001040 (University of Lausanne).

In particular, in case the kallikrein inhibitor is an inhibitor directed against hK2, said inhibitor can be selected among those disclosed in International Patent Application PCT/IB2004/001040, which content is incorporated herein by reference in its entirety. Preferably, the kallikrein inhibitor of the invention may be selected from the group comprising MD820, MD62, MD61, MD67 and MDCl. Most preferably this inhibitor is MD62 or MD61 and even more preferably the inhibitor is MDPK67b. This application discloses a chimeric inhibitor protein of a protease comprising an inhibiting polypeptidic sequence and at least one polypeptidic sequence of a substrate-enzyme interaction site specific for a protease as well as a method for producing the chimeric inhibitor protein of a protease. Preferably, the purified and isolated DNA sequence encoding the serine protease inhibitor of the invention is selected from the group comprising SEQ ID N° 1, SEQ ID N° 3, SEQ ID N° 5, SEQ ID N° 7, SEQ ID N° 9, SEQ ID N° 11, SEQ ID N° 13 and SEQ ID N° 15. The most preferably, the purified and isolated DNA sequence encoding the serine protease inhibitor of the invention is SEQ ID N° 15.

As an example of serine protease inhibitor according to the invention, Applicants have surprisingly found 6 new chimeric inhibitor proteins specific for the protease hK2 as resumed below in table II, these inhibitors are:

Table II

Chimeric inhibitors	Other name	SEQ ID N° (protein)
rACT _{8,20}	MD820	2
rACT _{6,2}	MD62	4
rACT _{8,3}	MD83	6
rACT _{6,7}	MD67	8
rACT _{6,1}	MD61	10
ACT _{5,18}	MD518	12
MDCI		14
MDPK67b		16

These chimeric inhibitor proteins have been obtained by modifying the RSL of α 1-antichymotrypsin (rACT), which is known to inhibit a large panel of human enzymes such as chymotrypsin, mast cell chymase, cathepsin G, prostatic kallikreins hK2 and PSA (hK3), in order to change the specificity of this serpin. Peptide sequences, selected as substrates for the enzyme hK2 by phage display technology as explained in International Patent Application PCT/IB2004/001040, have been used to replace the scissile bond and neighbor amino acid residues of the RSL. Recombinant inhibitors were produced in bacteria and purified by affinity chromatography.

Additionally, applicants have also found that replacing residues P3-P3' located in RSL structure of rACT_{WT} by substrate pentapeptide coding for the RSL of Protein C inhibitor (PCI) lead to the production of a chimeric inhibitor (MDCI) which is able to inhibit kallikreins hK2 and hK3.

In case the kallikrein inhibitor is an inhibitor directed against hK14, then said inhibitor can be selected among those disclosed in the priority International Patent Application PCT/IB2005/000504, which content is incorporated herein by reference in its entirety. Preferably, said recombinant inhibitor may be selected from the group comprising AAT_{G1}, AAT_{G1G}, AAT_{C11}, AAT_{C11G}, AAT_{E5}, AAT_{E8}, AAT_{F11}, AAT_{F3}, AAT_{G9}, ACT_{G1}, AcT_{G1G},

ACT_{C11}, ACT_{C11G}, ACT_{E5}, ACT_{E8}, ACT_{F11}, ACT_{F3}, ACT_{G9} (SEQ ID N° 17), ACT_{G1V}, and ACT_{C11D}. Preferably, said inhibitor protein of an hK14 protease is AAT_{G1}, AAT_{G1G}, AAT_{C11}, AAT_{C11G}, AAT_{E5}, AAT_{E8}, AAT_{F3}, AAT_{G9}, ACT_{G1G}, ACT_{C11}, ACT_{C11G}, ACT_{E5}, ACT_{E8}, AGT_{F11}, ACT_{F3}, ACT_{G9} (SEQ ID N° 18), ACT_{G1V}, or ACT_{C11D}. This application discloses a chimeric inhibitor protein of an hK14 protease having an inhibiting polypeptidic sequence and at least a polypeptidic sequence of a substrate-enzyme interaction site specific for said hK14 protease, wherein said chimeric inhibitor protein of an hK14 protease has, under physiological conditions,

- i) a stoichiometry of inhibition (SI) equal or below to 11.7 after at least 4 hours of incubation,
- ii) an association rate (K_a) of at least 7'500 M⁻¹ s⁻¹ ,
- iii) an inhibitory activity of 100% after at least 30 minutes of incubation.

In addition, the inhibiting polypeptidic sequence of the protease inhibitor may also be selected from a cysteine protease since there are now a number of well-documented instances of inhibition of cysteine proteases by serpins (*Gettins P.G.W.*, 2002 "Serpins structure, mechanism, and function" in *Chem. Rev*, 102, 4751-4803). These examples include inhibition of cathepsins K, L and S by the serpin squamous cell carcinoma antigen1, inhibition of prohormone thiol proteinase by the α -1-antichymotrypsin , and inhibition of members of the caspase family, including caspase 1 (interleukine 1 β converting enzyme), caspase 3, and caspase 8 by the viral serpin crmA and caspases 1, 4 and 8 by the human serpin PI9.

Also contemplated by the present invention are mixtures of serine protease inhibitors, antibodies, Peptabodies and biologically active fragments thereof.

Antibodies according to the invention can bind selectively a kallikrein or a serine protease and will not bind (or will bind weakly) to a non-target polypeptide. They can also bind to a naturally occurring kallikrein or serine protease or to recombinants polypeptide thereof. The antibodies of the invention can bind a kallikrein or serine protease expressed by a cell i.e. expressed by a cell includes cell-surface, membrane-bound, cytoplasmic or secreted forms. They can also bind one or more domains on the kallikrein or the serine protease, including the cytoplasmic, transmembrane, and/or extracellular domain(s). Alternatively, they can bind to any of the kallikrein or serine protease in their native and/or denatured forms.

It is understood by those skilled in the art that the regions or epitopes of the kallikrein or serine protease to which an antibody is directed can vary with the intended application.

The antibody according to the invention can recognize and bind any portion of the kallikrein or the serine protease, including the cytoplasmic domain, transmembrane domain, and/or the extracellular domain, or any portion thereof such as fragments or derivatives thereof.

Antibodies according to the invention can be polyclonal preparations which include a population of different antibodies directed against a different epitope on the immunogen, such as a kallikrein or serine protease used as an immunogen.

Polyclonal antibodies can be produced by methods well-known in the art. In general, any antibody (e.g., monoclonal, polyclonal, and the like) can be raised using an isolated kallikrein or a serine protease, or a fragment as the immunogen. In addition, the immunogen can be a fusion protein including all or a portion of the target polypeptides fused to V5, His, maltose-binding protein, GST, or human Ig. For example, polyclonal antibodies have been previously raised using a fusion protein having the extracellular domain of, for example, human hepsin fused to maltose-binding protein (Y Kazama, et al., 1995 J Biol Chem 270:66-72).

The antibodies according to the invention can be monoclonal antibodies that bind a specific antigenic site present on the kallikrein or the serine protease.

Methods for preparing an immunogen and immunizing an animal are well-known in the art (Kohler and Milstein 1975 Nature 256:495-497; Brown et al. 1981 J Immunol 127:539-46; Brown et al., 1980 J Biol Chem 255:4980-83; Yeh et al., 1976 Proc Natl Acad Sci USA 76:2927-31; Yeh et al., 1982 Int J Cancer 29:269-75; Kozbor et al., 1983 Immunol Today 4:72; Cole et al., 1985 Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96; U.S. Pat. No. 4,816,567; Clackson, et al., 1991 Nature 352:624-628; Marks, et al., 1991 J Mol Biol 222:581-597).

The present invention also envisioned the case where the Kallikrein inhibitors and/or the serine protease inhibitors are in the form of Peptabodies.

A "Peptabody" as disclosed in WO 98/18943 (Kajava et al.) and WO2004087766 (Université de Lausanne) which are incorporated herein by reference in their entirety, is a high avidity molecule which uses the multimerization concept for inducing aberrant cell signals. The

multimerization domain consists of a part of human cartilage oligomeric matrix protein (COMP), which is fused to a hinge region or spacer (preferably containing 19 amino acids from human IgA) and a domain (binding domain) capable of binding to an acceptor (ligand). The concept of peptabody molecule allows a tight binding on cells or tissues expressing high level of Kallikrein marker and serine protease. "Decabodies" are constructed on the same principle with the difference that they possess ten arms and consequently ten binding domains.

Usually, the diseases according to the invention are diseases in which the number of polymorphonuclear leukocytes, the neutrophils have become a problem by being decreased due to infections, septicemia, irradiation, chemotherapy, side effects of drugs or the action of toxic chemicals.

The invention also includes topical application of kallikrein inhibitors in diabetic skin ulcers to prevent cell death of neutrophils and thereby restore cellularity and functions of neutrophils.

The invention also includes the in-vitro use of kallikrein inhibitors or the serine protease inhibitors for preparation of neutrophils and their bone marrow precursors to perform molecular manipulations for gene therapy or to use neutrophils and their bone marrow precursors for infusions to patients.

The invention includes the treatment of patients receiving stem cells or myeloid precursor cells or neutrophil transfusions with kallikrein inhibitors or the serine protease inhibitors.

The present invention is also directed to a pharmaceutical composition comprising the kallikrein inhibitor and/or the serine protease inhibitor as described herein as an active agent, optionally in combination with one or more pharmaceutically acceptable carriers.

Preferably the composition, as a pharmaceutical composition, according to the invention is to be administered to a patient in need of treatment via any suitable route, usually orally or by injection into the bloodstream or CSF, or subcutaneously or directly into the site of interest, or close to this site.

Preferably, the composition according to the invention may also be added to infusion solutions prepared for infusions of bone marrow cells, myeloid cells and neutrophils.

According to another embodiment, the composition of the invention may also be added to solutions which are used in in-vitro manipulations of bone marrow cells and neutrophils for gene therapy or in cell freezing for storing of the cells.

According to a further embodiment, the composition of the invention may be applied locally to the skin in diabetic or ischemic skin ulcers.

The precise dose will depend upon a number of factors, including whether the composition is for prophylaxis or for treatment, the precise nature of the composition, and the nature of the detectable or functional label attached to the Kallikrein inhibitor or the serine protease inhibitor.

The present pharmaceutical composition comprises as an active substance a pharmaceutically effective amount of the composition as described, optionally in combination with pharmaceutically acceptable carriers, diluents and adjuvants.

“A pharmaceutically effective amount” refers to a chemical material or compound which, when administered to a human or animal organism induces a detectable pharmacological and/or physiologic effect.

The pharmaceutically effective amount of a dosage unit of the kallikrein inhibitor and/or the serine protease inhibitor as described herein usually is in the range of 0.001 ng to 100 µg per kg of body weight of the patient to be treated.

The pharmaceutical composition may contain one or more pharmaceutically acceptable carriers, diluents and adjuvants.

Acceptable carriers, diluents and adjuvants which facilitates processing of the active compounds into preparation which can be used pharmaceutically are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium

chloride, benzethonium chloride; phenol, butyl orbenzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

The form of administration of the pharmaceutical composition may be systemic or topical. For example, administration of such a composition may be various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, buccal routes or via an implanted device, and may also be delivered by peristaltic means.

The pharmaceutical composition, as described herein, may also be incorporated or impregnated into a bioabsorbable matrix, with the matrix being administered in the form of a suspension of matrix, a gel or a solid support. In addition the matrix may be comprised of a biopolymer.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and [γ] ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT(TM) (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished for example by filtration through sterile filtration membranes.

It is understood that the suitable dosage of the present composition will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any and the nature of the effect desired.

The appropriate dosage form will depend on the disease, the inhibitor, and the mode of administration; possibilities include tablets, capsules, lozenges, dental pastes, suppositories, inhalants, solutions, ointments and parenteral depots.

Since amino acid modifications of the amino acids (of the inhibitor for example) are also encompassed in the present invention, this may be useful for cross-linking the inhibitor to a water-insoluble matrix or the other macromolecular carriers, or to improve the solubility, adsorption, and permeability across the blood brain barrier. Such modifications are well known in the art and may alternatively eliminate or attenuate any possible undesirable side effect of the peptide and the like.

Usually, the Kallikrein inhibitors or the serine protease inhibitors of the invention can comprise a detectable label or can bind to a detectable label to form a detectable complex.

"Detectable labels" are detectable molecules or detection moiety for diagnostic purposes, such as enzymes or peptides having a particular binding property, e.g. streptavidin or horseradish peroxidase. Detection moiety further includes chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e. g. labelled avidin.

Preferably, detectable labels include fluorescent labels and labels used conventionally in the art for MRI-CT imaging. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow.

The Kallikrein inhibitors or the serine protease inhibitors of the invention may carry a radioactive label as the detection moiety, such as the isotopes ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{121}I , ^{124}I , ^{125}I , ^{131}I , ^{111}In , ^{211}At , ^{198}Au , ^{67}Cu , ^{225}Ac , ^{213}Bi , ^{99}Tc and ^{186}Re . When radioactive labels are used, known currently available counting procedures may be utilized to identify and quantitate the specific binding members. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

In the instance of *in vivo* imaging, the labels of the present invention may be conjugated to an imaging agent rather than a radioisotope(s), including but not limited to a magnetic resonance image enhancing agent. Examples of chelating groups include EDTA, porphyrins, polyamines crown ethers and polyoximes.

Examples of paramagnetic ions include gadolinium, iron, manganese, rhenium, europium, lanthanum, holmium and erbium.

Another subject matter of the present invention is to provide a kit for the diagnosis, prognosis, prophylaxis or treatment of neutropenia in a mammal, said kit comprising the composition of the invention, optionally with reagents and/or instructions for use.

The kit of the present invention may further comprise a separate pharmaceutical dosage form comprising for example an anti-cancer agent selected from the group consisting of chemotherapeutic agents, anti-epidermal growth factor receptors antibodies, radioimmunotherapeutic agents, and combinations thereof.

Generally, the Kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the condition of choice, such as neutropenia.

Alternatively, or additionally, the Kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The present invention also discloses the use of the composition of the invention, as a pharmacological tool in the development and standardization of *in vitro* and *in vivo* test systems for the diagnosis, prognosis, prophylaxis or treatment of neutropenia in mammals.

Also encompassed by the present invention is a detection assay for the diagnosis, prognosis, prophylaxis or treatment of neutropenia in a tissue sample comprising contacting the tissue sample with the composition of the invention, determining and measuring the amount of detected label and correlating this amount to the presence or absence of neutropenia in said tissue sample.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications without departing from the spirit or essential characteristics thereof. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features. The present disclosure is therefore to be considered as in all aspects illustrated and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety.

The foregoing description will be more fully understood with reference to the following Examples. Such Examples, are, however, exemplary of methods of practicing the present invention and are not intended to limit the scope of the invention.

Examples

MDPK67B in vitro effect on neutrophil cell survival.

To assess viability of neutrophils in-vitro, peripheral blood from healthy donors was erythrocyte-lyzed and neutrophils or peripheral blood mononuclear cells (PBMCs) were isolated. Cultures in RPMI10%FCS were performed in 96 well microtiter plates (5×10^5 cells/well) unless otherwise stated. The percentage of apoptotic neutrophils or PBMCs was assessed based on binding of fluorescent Annexin V- protein binding or measuring of CD11b or CD16 surface expression by FACS (fluorescent activated cell sorter) analysis.

Example 1: MDPK67b reduced apoptosis of neutrophils in vitro in a dose-dependent manner but has no significant effect on T-cell survival.

Figure 1: Annexin-V staining of neutrophils and T-cells upon incubation with protease inhibitors MDPK67b and MDOKG9.

Figure 1a: Annexin-V staining of neutrophils and T-cells upon incubation with MDPK67b. Cells were incubated for 24 or 48 hours with MDPK67b at concentrations ranging from 6 μ M to 60 μ M, as indicated, or PBS as control. Apoptosis was assessed by Annexin V staining and FACS analysis. Indicated leukocyte populations were gated based on their appearance in a forward scatter/ sideward scatter FACS dot plot (neutrophils) or by positive staining for CD3 (T cells).

Figure 1b: Annexin-V staining of neutrophils upon incubation with MDPK67b or MDOKG9 (OKDG9).

Neutrophils were incubated for 18 hours with MDPK67b or MDOKG9 concentrations ranging from 60 μ M (dilution 1) to 60 pM (dilution 7) as indicated. Apoptosis was assessed as outlined above.

Conclusion: MDPK67b at doses ranging from 60 μ M down to 0.6 μ M inhibit apoptosis of neutrophils. MDOKG9 had a similar effect protecting neutrophils entering apoptosis. This effect was specific to neutrophils and MDPK67B did not inhibit apoptosis of monocytes or lymphocytes.

Example 2: MDPK67b mediated protection of neutrophils against apoptosis is independent from culture conditions.

Figure 2: Comparison of various cell culture conditions through Annexin-V staining of MDPK67b treated neutrophils.

Neutrophils were cultured with the indicated concentrations of MDPK67b. PBS without MDPK67b served as a control. Neutrophils were plated (100 μ l/well) either at 5×10^6 /ml (high density) or 3×10^5 /ml (low density) and neutrophil apoptosis was assessed by AnnexinV staining and FACS analysis. Culturing of 5×10^6 /ml neutrophils in serum free medium (X-Vivo 15) instead of RPMI10% FCS was assessed in parallel.

Conclusion: MDPK67b inhibits apoptosis of neutrophils in vitro independently of cell density and presence or absence of serum in the growth medium.

Example 3: The Src tyrosine kinase inhibitor PP2 reverses MDPK67b mediated decrease in apoptosis of neutrophils.

Figure 3: Reversion of MDPK67b mediated neutrophil protection by tyrosine kinase inhibitors.

Figure 3a: Effect of MDPK67b on CD16 and CD11b levels of cultured neutrophils.

Neutrophils were cultured with the indicated concentrations of MDPK67b and percentage of neutrophils expressing high levels of CD16 or CD11b was assessed by FACS. Representative FACS plots are shown.

Figure 3b: Reversion of effect of MDPK67b on CD16 and CD11b neutrophils levels by PP2.

Neutrophils were cultured with the indicated concentrations of MDPK67b in presence or absence of the Src tyrosine kinase inhibitor PP2 (final concentration 10 μ M). Apoptosis and relative frequencies of CD11b and CD16 high expressing neutrophils were measured by FACS analysis.

Conclusion: MDPK67b increases dose-dependently the frequency of neutrophils that express CD16 and CD11b at high levels which is associated with decreased apoptosis. The increased frequency of CD11b high expressing neutrophils and the decreased apoptosis in the presence of MDPK67b can be reversed in the presence of the Src tyrosine kinase inhibitor PP2. Similar effects were observed with other kinase inhibitors blocking intracellular signaling pathways including the PI3K inhibitor Ly294002 and the ERK inhibitor PD98059.

Example 4: Superior effect of MDPK67b compared to G-CSF in protection of neutrophils from apoptosis

Figure 4: Effect of G-CSF on neutrophil in vitro apoptosis.

Neutrophils were cultured with the indicated concentrations of G-CSF and neutrophil Apoptosis (a) and down-regulation of CD16 expression (b) were analyzed by FACS. (c)

Neutrophils were cultured with MDPK67b (0.6 μ M) and titrated amounts of G-CSF (concentrations as indicated). Neutrophils cultured in medium and PBS (without MDPK67b) served as a control.

Conclusion: The effect of MDPK67b on neutrophil apoptosis is not affected by G-CSF which alone has only a mild protecting effect on neutrophil apoptosis.

Example 5: MDPK67b reduces cytostatic drug-induced apoptosis of neutrophils

Figure 5: Annexin-V and CD16 staining of neutrophils treated with MDPK67b and Etoposid.

Figure 5a: Annexin-V staining of neutrophils treated with MDPK67b and Etoposid.

Cells were incubated during 18 hours with MDPK67b (6 μ M) plus Etoposid (125 μ g/ml), Etoposid alone or PBS. Apoptosis was assessed by Annexin V staining and FACS analysis. Relevant leukocyte populations were gated based on their appearance in a forward scatter or sideward scatter FACS dot plot.

Figure 5b: Annexin-V staining of neutrophils treated with low MDPK67b and increasing Etoposid concentrations.

Cells were incubated for 18 hours with MDPK67b (0.06 μ M) alone or MDPK67b (0.06 μ M) plus increasing concentrations of Etoposid (in μ g/ml) as indicated or PBS. Apoptosis was assessed by Annexin V staining and FACS analysis was performed as mentioned above.

Figure 5c: CD16 staining of neutrophils treated with MDPK67b and Etoposid

Cells were incubated for 18 hours with MDPK67b (0.06 μ M) alone or MDPK67b (0.06 μ M) plus increasing concentrations of Etoposid (in μ g/ml) as indicated or PBS. Percentages of CD16 high expressing neutrophils were assessed by FACS analysis.

Conclusion: Even high doses (up to 125 μ g/ml) of the cytostatic drug Etoposid only partially block the apoptosis reducing effect of MDPK67b.

Example 6: RT-PCR Analysis of KLK expression in leukemic cell lines and donor derived mononuclear and neutrophil cells.

Material and Methods:

DU-145, PC-3, T47D, OVCAR-3, HL-60, THP1 and U937 cell lines were cultured in appropriate standard media with 10% deactivated fetal calf serum and incubated at 37 °C with 5% CO₂. Mononuclear and neutrophil cells were isolated. Total RNA was extracted from the cells using Trizol reagent (Life Technologies, Inc.) and PureLink Micro-to-Midi kit (Invitrogen) and two µg of total RNA were reverse-transcribed into first-strand cDNA using Superscript III (Invitrogen) in a 20-µl reaction following the manufacturer's instructions. PCR reactions were performed using specific primers for each kallikrein and actin primers as control. All primers were already described in literature (Harvey TJ et al., J Biol Chem, 2000 Dec 1;275(48):37397-406. Yousef GM et al., J Biol Chem. 2001 Jan 5;276(1):53-61. Yousef GM et al., Cancer Res. 2001 Apr 15;61(8):3425-31). Depending on the PCR reaction, RNA isolated from different cell lines including DU-145, PC-3, T47D, OVCAR-3 were used as positive controls for KLK expression (Harvey TJ et al., J Biol Chem, 2000 Dec 1;275(48):37397-406).

The cycling conditions were depending on the target gene and mainly as described in Harvey TJ et al., (J Biol Chem, 2000 Dec 1;275(48):37397-406). The PCR mixture was electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. Where indicated, DNA bands of the predicted size were excised from a second 2% agarose gel following electrophoresis and DNA recovered was sequenced.

Primers used for RT-PCR KLK amplification;

KLK	PRIMER	Sequence
KLK1	KLK1 F	TGGAGAACCACACCCGCCAAG
	KLK1R	ACGGCGACAGAAGGCTTATTG
KLK2	KLK2 F	GCCTAAAGAAGAATAGCCAGGT
	KLK2R	CTCAGACTAAGCTCTAGCACAC
KLK3	KLK3 F	GCATCAGGAACAAAAGCGTGA
	KLK3R	CCTGAGGAATCGATTCTTCAG
KLK4	KLK4F	GCGGCACTGGTCATGGAAAAGG
	KLK4R	CAAGGCCCTGCAAGTACCCG
KLK5	KLK5 F	GAGCTGGGGCCGGGGAAGAC
	KLK5R	TGGGCCGGGCACAAGGGTAA
KLK6	KLK6 F	GAGCGGCCATGAAGAAGC
	KLK6R	AATCACCATCTGCTGTCTTGC
KLK7	KLK7 F	GCCCAGGGTGACAAGATTATT
	KLK7R	GTACCTCTGCACACCAACGG
KLK8	KLK8 F	TACTCTGTGGCGGTGTCCTTG
	KLK8R	GAGCCCCAGGATGTGATGCCC
KLK9	KLK9 F	GGCCGGCCTCTTCCACCTTAC
	KLK9R	GCGCGGGCTCAGTTCTCCAT

KLK10	KLK10 F	GCGGAAACAAGCCACTGTGGG
	KLK10R	GGTAAACACCCCACGAGAGGA
KLK11	KLK11 F	CCGCTACATAGTTCACCTGG
	KLK11R	AGGTGTGAGGCAGGCGTAACT
KLK12	KLK12 F	TGGCAGACAAAGAGACAAGGT
	KLK12R	CTTAGAAGGGCTGGCAGGAG
KLK13	KLK13 F	CTACACCTGCTTCCCCCACTCTCA
	KLK13R	GCCGGTCAGGTTGCCACAT
KLK14	KLK14 F	CTGGGCAAGCACAAACCTGAG
	KLK14R	GCATCGTTTCCTCAATCCAGC
KLK15	KLK15 F	CAAGTGGCTCTCTACGAGCG
	KLK15R	ATCACACGGGTGGTCATGTG

Results:

Table 2: Expression patterns of the 15 KLK genes obtained by RT-PCR analysis in leukemic cell lines and donor derived mononuclear and neutrophil cells. The following symbols used represent: ++, moderate/high expression; +, low expression; (1) PCR products of the predicted size sequenced and confirmed to be the correct sequence.

	HL60	THP1	U937	Mononuclear	Neutrophil
KLK1	++	++ ¹⁾		++ ¹⁾	+ ¹⁾
KLK2	+	+	+ ¹⁾	+ ¹⁾	
KLK3					
KLK4					
KLK5				+	
KLK6					
KLK7					
KLK8			+		+
KLK9			++		
KLK10		+			
KLK11		+	+		
KLK12	+	+	+	+	++
KLK13	+ ¹⁾	++			
KLK14	++		+	++	
KLK15			+		

Conclusion:

RT-PCR analysis of KLK expression levels in leukemic cell lines and isolated human blood cells indicated that multiple KLKs are expressed and that the different cells have very diverse expression patterns for the KLK protease family. Such differences in KLK expression levels might be involved in different effects kallikrein inhibitors have on in vitro cultures of these cells as the described protection against apoptosis in neutrophil cells.

SEQUENCES LISTING**DNA Sequence ACT variants : MD 820**
SEQ ID N°1

ATGAGAGGATCC**CATCACCATCACCATCACT**CTAGACACCCTAACAGCCCACCTTGACGAGGA
GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCCTGTCTCTGGGGGCCCATATAAC
CACCTTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
TTCACCAGAGCTTCCAGCACCTCCTGCGCACCTCAATCAGTCCAGCGATGAGCTGCAGCTG
AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGAGGA
TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCAGAGACCCTGAAGCGGTGGAGAG
ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC
TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA
CCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAATCACCTCCGT
TCTCGAGCAGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT
CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variants : MD 820
SEQ ID N°2

MRGS**HHHHHH**SRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
IFSPLSISTALAFSLSLGAHNTTLTEILKGLKFNLTTETSEAEIHQSFQHLRLTNQSSDELQL
SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNGTRGKITDLIK
DLDSQTMVLVNYIFFKAKWEMPFPDQDTHQSRFYLSKKKWMVPMMSLHHLTIPIYFRDEEL
SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR
YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKITLR
SRAVETRTIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

Italic : start codon ATG

Bold : His-tag

Underlined : DNA mutation

Underlined and grey : DNA sequence encoding RSL mutation.

DNA Sequence ACT variant : MD 62
SEQ ID N°3

ATGAGAGGATCC**CATCACCATCACCATCACT**CTAGACACCCTAACAGCCCACCTTGACGAGGA
GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCCTGTCTCTGGGGGCCCATATAAC
CACCTTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA

TTCACCAGAGCTTCCAGCACCTCCTGCGCACCCCTCAATCAGTCCAGCGATGAGCTGCAGCTG
AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACACGGAGGA
TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCCAGAGACCCTGAAGCGGTGGAGAG
ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC
TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA
CCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAATCACCAGGAGG
TCTATCGATGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT
CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variant : MD 62
SEQ ID N°4

MRGS**HHHHHH**SRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
IFSPLSISTALAFSLSLGAHNTTLTEILKGLKFNLTETSEAEIHQS FQHLLRTL NQSSDELQL
SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIK
DLDSQTMMLVNYIFFKAKWEMPFPDQDTHQSRFYLSKKKWMVPMMSLHHLTIPYFRDEEL
SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR
YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKITRR
SID VETRTRIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA *

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA Sequence ACT variant : MD 83
SEQ ID N°5

ATGAGAGGATCC**CATCACCATCACCATCACT**CTAGACACCCTAACAGCCCCTTGACGAGGA
GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCTGTCTCTGGGGGCCATAATAC
CACCTTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
TTCACCAGAGCTTCCAGCACCTCCTGCGCACCCCTCAATCAGTCCAGCGATGAGCTGCAGCTG
AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACACGGAGGA
TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCCAGAGACCCTGAAGCGGTGGAGAG
ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC

TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGACCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGCTTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAAATCAGGGGGAGATCTGAGTTAGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGTCCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variant : MD 83
SEQ ID N°6

MRGSHHHHHHSRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNVIFSPLSISTALAFSLSLGAHNNTLTLEILKGLKFNLTTETSEAEIHQSFQHLLRTLNQSSDELQLSMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIKDLDSQTMMLVNYIFFKAKWEMPFPDQDTHQSRFYLSKKKWVMVPMMSLHHLTIPYFRDEELSCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISRDNYNLDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKIRGRSELVETRTRIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA Sequence ACT variant : MD 67
SEQ ID N°7

ATGAGAGGATCC**CATCACCATCACCATCAC**TCTAGACACCCTAACAGCCCACCTTGACGAGGA GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCCTGTCTCTGGGGGCCCATATAAC CACCCTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA TTCACCAGAGCTTCCAGCACCTCCTGCGCACCCCTCAATCAGTCCAGCGATGAGCTGCAGCTG AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGAGGA TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCCAGAGACCCTGAAGCGGTGGAGAG ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA CCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGCT TGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAAATCAAGCTTAGA ACAACATTAGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variant: MD 67
SEQ ID N°8

MRGSHHHHHHSRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
 IFSPLSISTALAFSLSLGAHNNTLLEILKGLKFNLTTETSEAEIHQSFQHLLRTLNLQSSDELQL
 SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIK
 DLDSQTMMLVNYIFFKAKWEMPFDPDQTHQSRFYLSKKKWVMPMMSLHHLTIIPYFRDEEL
 SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR
 YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKIKLR
TTLVETRTRIVRFNRPFLMIIVPTDTQNIFFMSKVTPKQA*

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA Sequence ACT variant : MD 61
SEQ ID N°9

ATGAGAGGATCC**CATCACCATCACCATC**ACTCTAGACACCCTAACAGCCCACCTTGACGAGGA
 GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
 ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
 ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCTGTCTCTGGGGGCCATAATAC
 CACCCTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
 TTCACCAGAGCTTCCAGCACCTCCTGCGCACCCCTCAATCAGTCCAGCGATGAGCTGCAGCTG
 AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGGAGGA
 TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
 AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
 GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
 GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
 TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
 TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
 TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCAGAGACCCTGAAGCGGTGGAGAG
 ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC
 TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA
 CCTGTGAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
 TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAATCATGACAAGA
TCTAACGCAGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT
 CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variant : MD 61
SEQ ID N°10

MRGSHHHHHHSRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
 IFSPLSISTALAFSLSLGAHNNTLLEILKGLKFNLTTETSEAEIHQSFQHLLRTLNLQSSDELQL
 SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIK
 DLDSQTMMLVNYIFFKAKWEMPFDPDQTHQSRFYLSKKKWVMPMMSLHHLTIIPYFRDEEL
 SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR

YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKIMTR
SNAVETRTIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA Sequence ACT variants : MD 518 SEQ ID N°11

ATGAGAGGATCC**CATCACCATCACCATCAC**TCTAGACACCCTAACAGCCCCTTGACGAGGA
 GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
 ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
 ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCCTGTCTCTGGGGGCCATAATAC
 CACCCTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
 TTCACCAGAGCTTCCAGCACCTCCTGCGCACCTCAATCAGTCCAGCGATGAGCTGCAGCTG
 AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGAGGA
 TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
 AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
 GACCTTGACTCGCAGACAATGATGGTCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
 GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
 TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
 TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
 TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCCAGAGACCCTGAAGCGGTGGAGAG
 ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC
 TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA
 CCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
 TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAAATCACCGAGCGT
GTCTCGCCCGTGGAGACGCGTACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT
 CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variants: MD 518 SEQ ID N°12

MRGSHHHHHHSRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
 IFSPLSISTALAFSLSLGAHNNTLTLEILKGLKFNLTETSEAEIHQS FQHLLRTL NQSSDELQL
 SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIK
 DLDSQTMMLVNYIFFKAKWEMPFDPDQTHQSRFYLSKKKWMVPMMSLHHLTIPYFRDEEL
 SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR
 YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKITER
VSPVETRTIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA Sequence ACT variants : MDCI
SEQ ID N°13

ATGAGAGGATCC**CATCACCATCACCATCAC**TCTAGACACCCTAACAGCCCACCTTGACGAGGA
GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCCTGTCTCTGGGGGCCATAATAC
CACCTTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
TTCACCAGAGCTTCCAGCACCTCCTGCGCACCTCAATCAGTCCAGCGATGAGCTGCAGCTG
AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGAGGA
TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTAGGGGGAAAATCACAGATCTGATCAAG
GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
TCAAGACAAGATGGAGGAAGTGGAAGCCATGCTGCTCCCAGAGACCCTGAAGCGGTGGAGAG
ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTCTACCTGCCAAAGTTTTCCATCTCGAGGGAC
TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTCACCAGCAAGGCTGA
CCTGTCAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCACCGCGGTCAAATCACC**TTAGA**
TCTGCATTAGTGGAGAC**CGGT**ACCATTGTGCGTTTCAACAGGCCCTTCCTGATGATCATTGT
CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Protein Sequence ACT variants: MD CI
SEQ ID N°14

MRGSHHHHHHSRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
IFSPLSISTALAFSLGLAHNTTLTEILKGLKFNLTTETSEAEIHQSFQHLRLTNQSSDELQL
SMGNAMFVKEQLSLLDRTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIK
DLDSQTMMLVNYIFFKAKWEMPFDPDQTHQSRFYLSKKKWVMPMMSLHHLTIPIYFRDEEL
SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISR
YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKIT**FR**
SALVETRTRIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

DNA sequence of MDPK67b
SEQ ID N°15

ATGCATCCGAACAGCCCGCTGGATGAAGAAAACCTGACCCAGGAAAACCAGGATCGCGGCAC
CCATGTGGATCTGGGTCTGGCCAGCGCGAACGTGGATTTTTCGTTTCAGCCTGTATAAACAGC
TGGTGCTGAAAGCGCCGGATAAAAACGTGATTTTTAGCCCGCTGTCTATTAGCACCGCGCTG
GCCTTTCTGAGCCTGGGCGCGCATAACACCACCCTGACCGAAATTCTGAAAGGCCTGAAATT
TAACCTGACCGAAACCAGCGAAGCGGAAATTCATCAGAGCTTTCAGCATCTGCTGCGTACCC
TGAACCAGAGCAGCGATGAACTGCAGCTGTCTATGGGCAACGCGATGTTTGTGAAAGAACAG
CTGTCTCTGCTGGATCGTTTTACCGAAGATGCGAAACGTCTGTATGGCAGCGAAGCGTTTGC
GACCGATTTTCAGGATAGCGCGGCGGCGAAAAAACTGATTAACGATTATGTGAAAAACGGCA
CCCGTGGCAAAATTACCGATCTGATCAAAGATCTGGATAGCCAGACCATGATGGTGCTGGTG
AACTACATCTTCTTCAAAGCGAAATGGGAAATGCCGTTTGATCCGCAGGATACCCATCAGAG
CCGTTTTTACCTGAGCAAAAAAAAATGGGTGATGGTGCCGATGATGAGCCTGCATCATCTGA

CCATTCCGTATTTTCGTGATGAAGAACTGAGCTGCACCGTGGTGGAACTGAAATATACCGGC
AACGCGAGCGCGCTGTTTATTCTGCCGGATCAGGATAAAATGGAAGAAGTGGAAAGCGATGCT
GCTGCCGGAAACCCTGAAACGTTGGCGTGATAGCCTGGAATTTCTGTGAAATTGGCGAACTGT
ATCTGCCGAAATTTAGCATTAGCCGCGATTATAACCTGAACGATATTCTGCTGCAGCTGGGC
ATTGAAGAAGCGTTTACCAGCAAAGCGGATCTGAGCGGCATTACCGGTGCGCGTAACCTGGC
CGTGAGCCAGGTGGTGCATAAAGCGGTGCTGGATGTGTTTGAAGAAGGCACCGAAGCGAGCG
CGGCGACCGCGGTGAAAATTAACTGCGTACCACCCTGGTGGAAACCCGTACCATTGTGCGT
TTTAACCGTCCGTTTCTGATGATTATTGTGCCGACCGATACCCAGAACATCTTTTTCATGAG
CAAAGTGACCAATCCGAAACAGGCGTAA

Amino acid sequence of MDPK67b
SEQ ID N°16

MHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNVIFSPLSISTAL
AFLSLGAHNNTTLTEILKGLKFNLTETSEAEIHQSFOHLLRLTNQSSDELQLSMGNAMFVKEQ
LSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNNGTRGKITDLIKDLDSQTMMLV
NYIFFKAKWEMPFDPDQTHQSRFYLSKKKWVMPMMSLHHLTIPYFRDEELSCTVVELKYTG
NASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSISRDNLDNDILLQLG
IEEAFTSKADLSGITGARNLAVSQVVHKAFLDVFEEGTEASAATAVKIKLRTLTVETRTIVR
FNRPFMIIVPTDTQNIFFMSKVTPKQA

Italic: start codon ATG

Bold: His-tag

Underlined: DNA mutation

Underlined and grey: DNA sequence encoding RSL mutation.

DNA sequence of ACT-G9 (alternative names: MDOKG9, OKDG9)
SEQ ID N°17

ATG**AGAGGATCCCATCACCATCACCATCACT****CTAGACACCCTAACAGCCC****ACTTGACGAGGA**
GAATCTGACCCAGGAGAACCAAGACCGAGGGACACACGTGGACCTCGGATTAGCCTCCGCCA
ACGTGGACTTCGCTTTCAGCCTGTACAAGCAGTTAGTCCTGAAGGCCCTGATAAGAATGTC
ATCTTCTCCCCACTGAGCATCTCCACCGCCTTGGCCTTCTGTCTCTGGGGGCCATAATAC
CACCCTGACAGAGATTCTCAAAGGCCTCAAGTTCAACCTCACGGAGACTTCTGAGGCAGAAA
TTCACCAGAGCTTCCAGCACCTCCTGCGCACCTCAATCAGTCCAGCGATGAGCTGCAGCTG
AGTATGGGAAATGCCATGTTTGTCAAAGAGCAACTCAGTCTGCTGGACAGGTTACGGAGGA
TGCCAAGAGGCTGTATGGCTCCGAGGCCTTTGCCACTGACTTTCAGGACTCAGCTGCAGCTA
AGAAGCTCATCAACGACTACGTGAAGAATGGAAGTGGGGGAAAATCACAGATCTGATCAAG
GACCTTGACTCGCAGACAATGATGGTCCTGGTGAATTACATCTTCTTTAAAGCCAAATGGGA
GATGCCCTTTGACCCCCAAGATACTCATCAGTCAAGGTTCTACTTGAGCAAGAAAAAGTGGG
TAATGGTGCCCATGATGAGTTTGCATCACCTGACTATACCTTACTTCCGGGACGAGGAGCTG
TCCTGCACCGTGGTGGAGCTGAAGTACACAGGCAATGCCAGCGCACTCTTCATCCTCCCTGA
TCAAGACAAGATGGAGGAAGTGGAGCCATGCTGCTCCAGAGACCCTGAAGCGGTGGAGAG
ACTCTCTGGAGTTCAGAGAGATAGGTGAGCTTACCTGCCAAAGTTTTCCATCTCGAGGGAC
TATAACCTGAACGACATACTTCTCCAGCTGGGCATTGAGGAAGCCTTACCAGCAAGGCTGA
CCTGTGAGGGATCACAGGGGCCAGGAACCTAGCAGTCTCCAGGTGGTCCATAAGGCTGTGC
TTGATGTATTTGAGGAGGGCACAGAAGCATCTGCTGCCAC**CGCGGTCAAAACCGTTGACTAC**
GCTGCTCTGGTGGAGACGCGT**ACCATTGTGCGTTTCAACAGGCCCTTCCCTGATGATCATTGT**
CCCTACAGACACCCAGAACATCTTCTTCATGAGCAAAGTCACCAATCCCAAGCAAGCCTAA

Italic and bold: start codon ATG

Bold and underlined: His-tag

Underlined: DNA mutation (added codon)

Underlined and grey: DNA sequence encoding RSL mutation.

Amino acid sequence of: ACT-G9 (alternative names: MDOKG9, OKDG9)
SEQ ID N°18

MRGS**HHHHHH**SRHPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSLYKQLVLKAPDKNV
IFSPLSISTALAFSLSLGAHNNTLTTEILKGLKFNLTTETSEAEIHQSFQHLLRTLNQSSDELQL
SMGNAMFVKEQLSLLDRFTEDAKRLYGSEAFATDFQDSAAAKKLINDYVKNGTRGKITDLIK
DLDSQTMMLVNYIFFKAKWEMPFDPDQTHQSRFYLSKKKWMVPMMSLHHLTIPYFRDEEL
SCTVVELKYTGNASALFILPDQDKMEEVEAMLLPETLKRWRDSLEFREIGELYLPKFSSISRD
YNLNDILLQLGIEEAFTSKADLSGITGARNLAVSQVVHKAVLDVFEEGTEASAATAVKTVDY
AALVETRTRIVRFNRPFLMIIVPTDTQNIFFMSKVTNPKQA*

Italic and bold: start Methionine

Bold and underlined: His-tag

Underlined: amino acid mutation (added)

Underlined and grey: RSL mutation

Claims

1. A method for the treatment or prevention of patients suffering from neutropenia comprising the administration to said patients in need thereof of a therapeutically effective amount of serine protease inhibitors.
2. The method of claim 1, characterized in that the serine protease inhibitors is a Kallikrein inhibitor.
3. The method of claim 2, characterized in that the Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof.
4. The method of claim 3, characterized in that the Kallikrein inhibitor is selected among hK2, hK4, hK11, hK5, hK14 inhibitors or mixtures thereof.
5. The method of claim 4, characterized in that the Kallikrein inhibitor is an hK2 inhibitor.
6. The method of claim 1, wherein the serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.
7. Serine protease inhibitors for use in a method of treating or preventing neutropenia in patients which develops due to infections, septicemia, chemotherapy, irradiation, toxic chemicals or as side effects of any medication.
8. Serine protease inhibitors for use in a method of treating or preventing neutropenia of claim 7, wherein the number and / or activation state of neutrophils is impaired.
9. Serine protease inhibitors of claim 8, for use in a method of treating or preventing skin ulcers in diabetes patients in which neutrophils undergo cell death, or skin ulcers developing in patients with peripheral arterial disease associated with hypoxic conditions in the skin and neutrophil dysfunction and apoptosis.

10. Serine protease inhibitors of claim 7, for use in a method of treating or preventing irradiation induced damage of myeloid cells as occurs in the course of treatment of malignancy, accidents in nuclear plants or use of nuclear weapons.
11. Serine protease inhibitors of claims 7-10, wherein the serine protease inhibitors is a Kallikrein inhibitor.
12. Serine protease inhibitors of claim 11, characterized in that the Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof.
13. Serine protease inhibitors of claims 7-12, wherein the serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.
14. Serine protease inhibitors for use in the *in-vitro* preparation of neutrophils and their bone marrow precursors
 - to perform molecular manipulations for gene therapy prior to infusion of myeloid cells to patients with neutropenia or genetic disorders of the myeloid system,
 - or to use neutrophils and their bone marrow precursors for infusion to patients with neutropenia or dysfunction of neutrophils.
15. The serine protease inhibitors of claim 14, wherein the serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.
16. A method for the prevention of apoptosis of myeloid cells of patients, comprising the administration to said patients in need thereof of a therapeutically effective amount of serine protease inhibitors:
 - (1) during and after transfection of bone marrow cells performed for gene therapy,
 - (2) during blood stem cell mobilization performed for reconstitution of hematopoiesis and/or

(3) during infusion of cells of the myeloid lineage for reconstitution of hematopoiesis for gene therapy or for treatment of neutropenia by infusion of neutrophils.

17. The method of claim 16, wherein the serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.

18. A kit for the diagnosis, prognosis, prophylaxis or treatment of neutropenia in a mammal, characterized in that said kit comprises serine protease inhibitors, optionally with reagents and/or instructions for use.

19. The kit of claim 18, wherein the serine protease inhibitors comprise a detectable label or can bind to a detectable label to form a detectable complex.

20. The kit of claims 18-19, wherein the serine protease inhibitors is a Kallikrein inhibitor.

21. The kit of claim 20, wherein in the Kallikrein inhibitor is selected amongst hK2, hK3, hK4, hK5, hK6, hK7, hK8, hK9 hK10, hK11, hK12, hK13, hK14, hK15 inhibitors or mixtures thereof.

22. The kit of claims 18-21, wherein the serine protease inhibitors are selected from the group comprising SEQ ID N° 2, SEQ ID N° 4, SEQ ID N° 6, SEQ ID N° 8, SEQ ID N° 10, SEQ ID N° 12, SEQ ID N° 14, SEQ ID N° 16, SEQ ID N° 18 or mixtures thereof.